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Experimental infection of calves with Mycoplasma dispar

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Experimental infection of calves with *Mycoplasma dispar*

by

Olimpio Crisostomo Ribeiro

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INTRODUCTION

Calf pneumonia has long been a worldwide problem in both dairy and beef herds. Its etiology has been repeatedly proven to be complex in nature. Under intensive conditions, physical stresses are generally accepted as being of special significance in addition to infectious agents. Evidence is also accumulating in the literature which indicates that the severe clinical disease seen under field conditions is often the result of interactions of infectious agents under the influence of physical stress. With this in mind, it is important to improve understanding of any isolated agent which appears to be a relevant partner in this complex group of diseases.

Several mycoplasma species have been added in the last few years to the long list of agents alleged to play some role in calf pneumonia. However, experimentation to prove the mycoplasma involvement in bovine respiratory disease is still scarce, especially in North America.

The ubiquity of Mycoplasma dispar in the cattle population as indicated by several recent reports prompted its inclusion in the research project on bovine respiratory disease at the Iowa State University Veterinary Medical Research Institute. This particular experiment was planned to study the role of M. dispar in lung lesions of normal and stressed calves, to determine its approximate incubation period and to obtain information on its pathogenetic patterns.

To approach the proposed objectives, a series of four experiments was conducted during the Summer and early Fall of 1978. These four

experiments utilized 24 calves which were assigned to two basic treatments: (1) dexamethasone injections for a period of 10 to 12 days and (2) challenge with M. dispar. Calves were treated with none, one, or both of these treatments for a total of four different groups. A group of seven animals was treated with both dexamethasone and M. dispar, seven were treated with M. dispar only, five were treated with dexamethasone only, and five were given saline and served as controls. Calves from these groups were necropsied at 11, 12, 16, 20 and 21 days after inoculation to observe infection with M. dispar and to evaluate lesions.

LITERATURE REVIEW

Bovine respiratory diseases have been a favored topic for research in recent years. Many American universities and other research centers throughout the world have specific research projects on this area. The respiratory disease of cattle has been recognized as a broad and complex subject. Hence, a useful way of viewing the problem is by separating those conditions mainly affecting adult cattle from those primarily seen in calves since they supposedly have differences in etiology and pathogenetic mechanisms. This fact has been emphasized by a series of reports. Breeze et al. (1975), Selman et al. (1977) and Breeze et al. (1978) have reviewed the different aspects of conditions that are mainly prevalent in adult cattle. Pirie (1977) reviewed a series of distinct conditions of both adult and young animals. There are also several reviews dealing purely with the economically important calf pneumonia complex (Kenzy, 1948; Jarrett, 1956; Omar, 1966; Ide, 1970; Gourlay et al., 1970; Bitsch et al., 1976; Bryson et al., 1978a, 1978b). The agents claimed to be involved have increased in nature and number to include a variety of different groups of microorganisms, parasites and even chemicals. The above reviews have indicated that researchers have throughout the years isolated more than 10 different genera among the bacterial agents, more than a dozen different viruses and several species of mycoplasmas, fungi and helminths. Even though they have on occasion been suggested as etiologic agents, very few, if any, of them have in fact been proven to play a primary or definite role.

Among the bacteria, Pasteurella multocida and Pasteurella hemolytica are probably the ones of major concern in so-called shipping fever complex because they have been the most consistently isolated from fibrinous bronchopneumonia which is characteristic of shipping fever. Omar (1966) points out that, in all cases of pasteurellosis, the fibrinous exudate pervades not only the alveoli but also the interlobular septa, giving rise to a marbled appearance of the lungs, not unlike the gross lesions of contagious bovine pleuropneumonia. Details of the histopathology of both the catarrhal and fibrinous types of exudative bronchopneumonia are described by Jubb and Kennedy (1970). Tentative guidelines for correlating the pathological findings with the species of Pasteurella isolated are found in a recent report (Schiefer et al., 1978). The correlation between other organisms and lesions is given in a later report (Bryson et al., 1978b).

The list of viruses as reviewed by Mohanty (1978) is composed of six different families, namely Herpetoviridae, Adenoviridae, Paramyxoviridae, Togaviridae, Reoviridae and Picornaviridae. The different viruses within eight genera of these 6 families amount to at least 26 species or serotypes. Many of these were also listed in previous reviews (Omar, 1966; Thomas, 1973; Potgieter, 1977).

Mycoplasmas have in the last few years been proven to be the etiologic agents of diseases of several animal species including the bovine species. It should be mentioned that as many as 13 mycoplasma species have already been found in cattle (Leach, 1967, 1973; Langford et al., 1976; Gourlay et al., 1977). Some of them have been recovered

from one or more particular tissues where an association with observable lesions was reported and some were found without a definite correlation with lesions. In the bovine respiratory tract, in particular, a few mycoplasma species, namely Mycoplasma bovis, Mycoplasma dispar, Mycoplasma bovirhinis and Ureaplasma have been strongly suggested as playing a definite role in pathologic processes.

The remainder of this section will be a review of the pertinent literature on calf pneumonia with emphasis on the involvement of mycoplasmas, particularly M. dispar, as they relate to that group of cattle respiratory diseases.

Calf pneumonia has long been a worldwide problem in both dairy and beef herds. Many early researchers either mentioned or did some work on this subject (Nocard, 1901; Smith, 1918, 1921 and 1925; Carpenter and Gilman, 1921; Jones and Little, 1921 and 1922; Hallman et al., 1928; Ray, 1928; Roberts, 1938; Thorp and Hallman, 1939; Thorp et al., 1942; Kenzy, 1948). Many different species of bacteria were isolated by those researchers and were considered to be associated with observable lesions in most instances. Some of these bacteria have later been confirmed to play a definite role and some have been discarded as primary agents.

Kenzy (1948) stated that during the period 1939 to 1942 the incidence of calf pneumonia reached epizootic proportions in Iowa. The 1939-1942 outbreaks served as a source of materials for his studies on calf pneumonia. He studied microbiology and pathology in animals with pneumonia which were submitted to the Iowa State University Veterinary Diagnostic Laboratory. His literature review discussed in detail the works of

most of the early researchers which were listed by a later extensive and comprehensive review (Omar, 1966) as original and authoritative on each particular agent. Many different bacteira and an unknown virus were included. Several of those early reports presented both gross and microscopic lesions in some detail (Thorp and Hallman, 1939; Thorp et al., 1942; Jennings and Glover, 1952; Jarrett, 1956; Jarrett et al., 1953, 1954). A multiplicity of predisposing factors had already been incriminated by research workers as pointed out in both Kenzy's and Omar's reviews. Kenzy (1948) was one of the first researchers to draw attention to the economic importance of calf pneumonia in Iowa herds. Kenzy's own results gave a useful description of the bacteria isolated and of both gross and light microscopy observations.

Kenzy (1948) found several genera of bacteria involved in the Iowa outbreaks. His list included Pasteurella, Corynebacterium, Alcaligines, Pseudomonas and Actionomyces, but he only attributed importance to the first two genera. Lesions described in association with P. multocida and P. hemolytica were bilateral bronchopneumonia with consolidation of cranial lobes and anteroventral portions of the diaphragmatic lobes. There was a suppurative bronchiolitis involving small sectors of the bronchiolar walls. A serofibrinous exudate was described in the alveoli. The pleura and the interlobular septa had a serofibrinous exudate. Corynebacterium spp. were associated with an acute bronchitis, a suppurative bronchiolitis and a marked dilation of the interalveolar vessels, along with some of the pasteurella associated lesions. Many

years later Ramsey et al. (1968) also expressed their concern about the multiplicity of agents involved in bovine respiratory diseases as they were observed in Iowa herds and the problems involved in reaching an accurate diagnosis.

Harbourne et al. (1965) mentioned an apparent rise in the incidence of calf pneumonia in England in the early 1960's which lead to a number of research papers and correspondence (mainly directed to The Veterinary Record) regarding pneumonia in calves. Soon after that, Omar (1966) wrote his extensive and comprehensive review, which has been a valuable reference. Since then there seems to have been an increased concern about pneumonia of calves and about bovine respiratory disease in general.

Jennings and Glover (1952) mentioned that there had been several studies of the types of bacteria recovered from the lungs of calves, but that there was no clear evidence which would incriminate a bacterium as the primary agent in pneumonia. They were able to produce a low-grade pneumonia in experimental calves, using filtered and unfiltered material, obtained from field cases of pneumonia, and they concluded that the responsible agent was of the nature of a virus.

Paterson (1962) has pointed out, however, that one form of the calf pneumonia, previously described by Jarrett (1956) and Jarrett et al. (1953, 1954) as "cuffing" pneumonia, has much in common with so-called virus pneumonia or enzootic pneumonia of pigs, grey lung disease of mice and Eaton's atypical pneumonia of man. The evidence available at that time indicated that all those conditions might be due to mycoplasma or mycoplasma-like agents. The Eaton agent has now been

positively identified as a mycoplasma (Purcell and Chanock, 1967; Gusman, 1974; and Stanbridge, 1976). Enzootic pneumonia of pigs has also been proven to be caused by mycoplasma (Betts and Whittlestone, 1963; Goodwin and Whittlestone, 1963, 1964; Goodwin et al., 1965; Lutsky and Organick, 1966). A few reports on pathogenesis (Livingston et al., 1972 and Mebus and Underdahl, 1977), on pathogenicity (Ross, 1973) and on pathology (Jericho, 1977) further emphasize the role of mycoplasmas in enzootic pneumonia of pigs.

Mackay et al. (1963) have reported the isolation of mycoplasmas from cases of ovine pulmonary adenomatosis. The role of mycoplasmas in respiratory tract diseases of both sheep and goats has been emphasized by recent reports (Ojo, 1977; Foggie et al., 1976; Ernø et al., 1978). The dog has also been the subject of experimental mycoplasma pneumonia (Rosendal and Vinther, 1977).

Isolations of mycoplasmas from a variety of other animal species and their possible correlation with the presence of disease processes are reviewed elsewhere (Maramorosch, 1973; Freundt, 1974; Taylor-Robinson, 1975; Timms, 1978). Diagnosis of bovine pleuropneumonia, including pathological diagnosis, has been recently reviewed (Windsor, 1976).

A number of different mycoplasma species or serotypes have been isolated from bovine respiratory tracts, genital tracts, mammary glands, joints, eyes and even from the digestive tract. The publications were often a single report of the isolant, but pathogenicity and biological behavior have also been investigated in many instances (Carter, 1954a,

1954b; Carter and McSherry, 1955; Hamdy et al., 1958; Olson et al., 1960; Langer and Carmichael, 1963; Harbourne et al., 1965; Dawson et al., 1966; Davies, 1967; Ernø, 1967; Gourlay, 1968, 1969; Ernø, 1969; Ernø and Philipsen, 1969; Gourlay and Thomas, 1969; Hirth et al., 1970; Ide, 1970; Gourlay and Thomas, 1970; Gourlay et al., 1970; Ernø, 1971; Jurmanova and Krejci, 1971; Ernø and Aalund, 1972; Thomas and Smith, 1972; Ernø and Blom, 1972, 1973; Blom et al., 1973; Frey, 1973; Gourlay, 1973; Shimizu et al., 1973; St. George et al., 1973; Ernø, 1975; Gourlay et al., 1975; Langford, 1975; Ose and Muenster, 1975; Mensik et al., 1975; Pirie and Allan, 1975; Thomas et al., 1975; Shimizu et al., 1975; Allan et al., 1976; Brownlie et al., 1976; Gourlay et al., 1976a; Howard et al., 1976; Oghiso et al., 1976; Bennett and Jasper, 1977a, 1977b, 1978; Bennett et al., 1977; Dellinger et al., 1977; Kuniyasu et al., 1977; Langford, 1975, 1977; Kishima et al., 1978; Bryson et al., 1978a, 1978b). There is even a review on pathogenicity of bovine mycoplasma (Fabricant, 1973).

Such a list of scientific reports which come from many different countries indicates a wide distribution of many mycoplasma species in the cattle population. However, as pointed out by Gourlay (1973), in only 4 naturally occurring bovine diseases have mycoplasmas been proven to be of primary etiologic significance. The diseases are contagious bovine pleuropneumonia caused by Mycoplasma mycoides, mastitis caused by M. bovis, mastitis caused by Mycoplasma bovigenitalium, and arthritis caused by M. bovis. As far as the bovine respiratory tract is concerned, a number of reports (including several of the above mentioned) are now

available in the literature dealing with the involvement of mycoplasmas, other than M. mycoides, in pathologic processes. Carter (1954a and 1954b) and Carter and McSherry (1955) in Canada recovered several strains from nasal swabs and lung tissue of animals with shipping fever. Three strains were isolated from similar cases in the USA by Hamdy et al. (1958). They attempted unsuccessfully to infect three calves with these three strains. One calf was inoculated intratracheally with 4 ml and intranasally with 6 ml of a combined suspension of the three strains. A second calf was inoculated similarly with half that dosage plus similar quantities of P. multocida and a third one was inoculated exactly as was calf 2 and, in addition, was given 0.5 g of cortisone acetate intramuscularly as a stressing agent. The cortisone acetate was given the day before and again at the time of exposure to the cultures. They concluded that a pure culture of mycoplasma, alone or with P. multocida, was not capable of producing the syndrome of shipping fever in calves and that the organisms probably were pathogenic but that other synergistic agents were required.

In the United Kingdom, Harbourne et al. (1965) isolated mycoplasma from 10 pneumonic lungs and 46 nasal swabs of calves with a respiratory disease. These isolates were classified into two groups. Organisms of group B, consisted only of nasal strains and had characteristics of the non-pathogenic Mycoplasma laidlawii. The others, group A, belonged to an unknown serotype. The pathogenicity of these organisms was not tested in calves. It was reported, however, that serological examination of paired sera revealed a somewhat higher agglutinating antibody

titer against the group A serotype in calves from two farms where the strains had been recovered than in animals on another farm where the organism was not isolated.

Gourlay and Thomas (1969) communicated that they established infection following inoculation with the T-strains and the atypical strains previously recovered by Gourlay (1968, 1969). However, detailed description of the disease was not reported until later (Gourlay and Thomas, 1970).

Starting in the early 1960's and continuing for the whole decade a number of workers succeeded in isolating mycoplasmas from bovine respiratory tracts in both healthy and diseased animals (Olson et al., 1960; Langer and Carmichael, 1963; Harbourne et al., 1965; Dawson et al., 1966; Davies, 1967; Leach, 1967; Gourlay, 1968, 1969). Most of these strains were typical large colony mycoplasmas. Gourlay's strains, however, were reported to be T-mycoplasmas (Gourlay, 1968) and a group of mycoplasmas considered to be atypical because they required special media for growth (Gourlay, 1969). Researchers then became aware of the ubiquity of mycoplasmas in the upper respiratory tract of normal cattle and in the lungs of calves with pneumonia, but little more than isolation was actually done.

As the 1970's started, a growing concern about all aspects of mycoplasmology became apparent. Several researchers pursued the subjects of characterization, classification and disease etiology. Gourlay and Leach (1970) characterized and fully described the atypical strains previously recovered by Gourlay (1969) and concluded that all were a

single species which they named M. dispar. A further comparative study was conducted by Friis (1978) who utilized 70 strains of M. dispar recovered from pneumonic tissue of calf lungs in Denmark. Friis pointed out that there seemed to be some degree of heterogeneity among isolates of M. dispar. An extensive characterization study was conducted by Leach (1973) on a large number of previously isolated bovine strains. These strains were not only compared with each other but also compared with almost all of the named mycoplasma species from non-bovine sources. This work confirmed previous characterization studies but yet had the merit of ending a dual classification scheme as suggested by Leach (1967) and Al-Aubaidi and Fabricant (1971). Thus, almost all of the bovine mycoplasmas were specifically characterized and named. This ended the confusion that had been caused by the existence of two essentially similar classification schemes for bovine mycoplasmas which had different systems of nomenclature (Leach, 1967; Al-Aubaidi and Fabricant, 1971). The methods of characterization themselves and diagnostic procedures as applied to bovine mycoplasmas have paralleled the tremendous improvements of general mycoplasmology (Al-Aubaidi and Fabricant, 1971; Ernø, 1972, 1977; Leach, 1973; Ernø and Stipkovits, 1973a, 1973b; Ernø and Jurmanova, 1973; Ernø et al., 1973; Howard and Gourlay, 1974; Cho et al., 1975; Schmitz and Gradin, 1975; Howard and Gourlay, 1974; Stalheim et al., 1975; Allan et al., 1976; Bitsch et al., 1976; Stalheim, 1976; Allan and Pirie, 1977; Dellinger et al., 1977; Hill, 1978; Thomas and Hidalgo, 1978).

Suitable diagnostic procedures have been outlined and recommended by the Mycoplasmosis Committee of the American Association of Veterinary Laboratory Diagnosticians (Stalheim, 1976). New techniques are continuously being developed. These improvements have encouraged diagnosticians and researchers toward field and experimental observations on bovine mycoplasmosis in many different countries as indicated by the above references. The Iowa State University Veterinary Diagnostic Laboratory and the Department of Veterinary Pathology have in the last few months submitted pneumonic lungs from about 40 calves to be examined for mycoplasmas at the Iowa State University Veterinary Medical Research Institute. At least one-half of them were positive for single or multiple infections with M. dispar, M. bovis, M. bovirhinis, M. bovoculi, or Ureaplasma spp. (W. U. Knudtson, Iowa State University Veterinary Medical Research Institute, personal communication).

Following are details of reports dealing with microbiology, pathology and some other aspects of field and experimental cases of mycoplasma infections, especially in regard to M. dispar in the bovine respiratory tract.

Microbiological and pathological observations were made in 65 pneumonic calf lungs from both clinically healthy veal calves and calves that had died or been killed in extremis (Gourlay et al., 1970). Mycoplasmas were the most frequently isolated microorganisms and were isolated from 75% of these lungs. T-mycoplasmas or Ureaplasma spp. as they were later named by Shepard et al. (1974) were isolated from 58.5 percent, Mycoplasma dispar from 51 percent and Mycoplasma bovirhinis from 23 percent of the lungs. A variety of lesions were described. These included

peribronchial lymphoid hyperplasia as seen in cuffing pneumonia (Jarrett et al., 1953, 1954; Omar, 1966). Peribronchial lymphoid hyperplasia was observed in 75 percent of the lungs from the "healthy" calves but in only 5 percent of the lungs of the calves that died. This difference lead the authors to indicate that peribronchial lymphoid hyperplasia and mycoplasma may not be associated, though other explanations would be possible. The high incidence of Ureaplasma spp. and M. dispar raised the question of their role in calf pneumonia, and in a preliminary communication (Gourlay and Thomas, 1969) it was reported that both could produce experimental pneumonia when inoculated endobronchially into conventionally reared calves. Later, in a more detailed study (Gourlay and Thomas, 1970), ureaplasmas were inoculated and clinical signs of pneumonia were observed in six of 16 calves before slaughter and pneumonic lesions were found in 14 of them. Ureaplasmas were isolated from 13 of the 16 lungs. Histological examination of the lungs revealed acute bronchiolitis associated with alveolar collapse. The relative absence of peribronchiolar lymphoid hyperplasia was tentatively ascribed to the short interval between inoculation of the calves and their slaughter.

Data obtained by Thomas and Smith (1972) suggest that calves may be infected by M. dispar during the first day of life. In a survey of apparently healthy one to two-day-old calves, they were able to isolate the organism from a few calves from several levels of the respiratory tract in decreasing frequency from the nose to the lung. In another age group (three to four-month-old calves), however, they found no

M. dispar in the nose, but found it much more frequently in the trachea, bronchus and lung. In a still older group the organism was again found in the nose and trachea but not in the bronchi or in the lung. They reported as principal findings that non-pneumonic calves in the 3 to 4 months age group had active colonization of all parts of the respiratory tract. Large numbers of mycoplasmas were found in the actual lung tissue in contrast to the very young calves which appeared to harbor mycoplasmas only in small numbers mainly in the nasal cavity. Older animals, 10 or more months, also carried a low burden of mycoplasmas mainly in the upper respiratory tract. They suggested that as the calf grows older, mycoplasmas progressively colonize the respiratory tract until some point between 4 and 10 months of age when they decline. Their data are particularly significant because of their choice of age groups when monitoring herds and because they also contributed to further pathogenetic studies. It is unfortunate, however, that this work did not include the histologic appearance of the respiratory tract.

Gourlay and Leach (1970) and Gourlay et al. (1970) reported that cultural examination of 20 healthy calf lungs and 10 healthy cow lungs failed to reveal mycoplasmas. Age range and cultural method differences between their experiments and those of Thomas and Smith (1972) may have been responsible for apparently contradictory results. Thomas and Smith (1972) claimed that they would, in fact, be comparable, for in both their youngest and oldest groups, no evidence of colonization by mycoplasma in the lung tissue was found. They also claimed that M. dispar and M. bovirhinis, as well, are only slightly higher in number

and frequency than the isolations from comparable normal animals in the experiment of Gourlay and his colleagues.

By collecting calves in different batches and from different sources, St. George et al. (1973) studied a continuous population of 52 calves. They were killed at different ages and at different times but they were grouped into specific age ranges. They had 3 age groups in which the results were recorded. Seventy one percent of their series had macroscopic abnormalities of the lungs. The most common gross lesions were reported as dark lobular areas of collapse (atelectasis) usually encompassing several lobules but variable in size from 2 or 3 mm to 4 or 5 cm. They found a greater severity in animals 7 to 16 weeks of age than in those making up the 1 to 6 or 20-week-old groups. They also found a far greater frequency of lesions in the right lung lobes (86%) than in the left ones (38%). Interstitial thickening and alveolar collapse were reported as constant features in all age groups. These lesions were actually noted in the lungs of all but 2 calves, one of which was 2 weeks old and the other 4 weeks old. They found the lesions to be lobular in distribution and areas where hyperplasia of interstitial cells had progressed to lobular collapse corresponded to macroscopic lesions. They observed that there were age-related differences in the microscopic findings. Interstitial thickening and alveolar collapse were most marked in younger calves (1 to 6 weeks). A diagnosis of proliferative interstitial pneumonia was made in some of these calves (4) on the basis of considerable evidence of interstitial proliferative activity. An additional

feature to the above was proliferative dysplastic change in bronchiolar epithelium. There was only limited evidence of peribronchial lymphoid hyperplasia. Their 7 to 16 week group was reported to have the most pronounced microscopic lesions. Peribronchial and perivascular lymphoreticular hyperplasia together with purulent bronchiolitis constituted their main findings. They also found that lobular atelectasis could sometimes be related to bronchioles occluded by plugs of neutrophils or compressed by hyperplastic aggregations of lymphoid tissue. Atelectatic areas were occasionally lightly infiltrated by neutrophils.

The microscopic changes of the 20-week-old calves were relatively mild. The authors suggested that the lesions had regressed by this time. Bronchiolitis and lymphoid hyperplasia were again the main findings. There was slight interstitial thickening and atelectasis. M. dispar was isolated more frequently from the 7 to 16-week old group as well.

St. George and his colleagues in the same paper also reported the results of transmission studies conducted in seven-day-old caesarean derived and colostrum deprived calves. Two calves served as controls and were given sterile mycoplasma medium intratracheally. Three of the infected calves became moribund without exhibiting respiratory signs. The controls remained normal. They described lobular areas of atelectasis, distributed through all pulmonary lobes, and microscopic lesions of proliferative interstitial pneumonia in infected animals. There was interstitial cell proliferation together with lobular and sublobular atelectasis. Areas with atelectasis were lightly

infiltrated by neutrophils and increased numbers of alveolar macrophages in some areas. Perivascular and peribronchial lymphoid hyperplasia were only mild. Small and terminal bronchioles had proliferative dysplastic epithelial changes, but contained only small numbers of leukocytes. They found that no gross lung lesions were observed in control calves but interstitial thickening along with interstitial cell hyperplasia and proliferation was observed. There were also lymphoreticular hyperplasia along inter-lobular septa and perivascular lymphoid aggregates in a few lobules in the control calves. The number of alveolar macrophages was increased in some lobules.

Their general conclusions were that there were generalized histopathological changes in age-series calves which were not confined to macroscopically abnormal areas even though these had the greatest degree of lobular collapse. They also concluded that lungs assessed as normal had marked histological changes.

Thomas and Howard (1974) studied the effect of several strains of different species of mycoplasmas in bovine fetal tracheal explant cultures. They concluded that all those strains studied multiplied in the explant cultures, but only M. dispar produced cytopathic effects on the ciliated epithelial cells and caused progressive sloughing of cells and patchy flattening of the epithelial layer after 6 days. The cytopathic effect was associated with the presence of large numbers of mycoplasmas.

The involvement of T-mycoplasmas in calf pneumonia was reported by Shimizu et al. (1975) who described their observations on 22

pneumonic calves and reviewed previous reports related to the same kind of infections. Fifteen of the 22 calves yielded Ureaplasma spp. The organism was also recovered from tracheas of 9 of the 22 calves. They examined 43 calves and beef cattle without pneumonia. None of these harbored a detectable number of T-mycoplasmas (Ureaplasma spp.) The report included no histopathologic evaluations other than listing interstitial pneumonia, abscess formation, exudation of fibrin and bronchial exudates. Gross lesions appeared to be extensive involving cranial lobe, and in some instances extending through the caudal lobes. The authors suggested that T-mycoplasma may be more closely related to calf pneumonia than any mycoplasma species, even though they were able to demonstrate low titers of M. bovirhinis along with T-mycoplasmas in some of the lungs. They considered M. bovirhinis as an opportunist invader.

Pirie and Allan (1975) studied the relationship between mycoplasmas and the pulmonary lesions of 20 calves which were reared together for 6 months, beginning after their first week of life, when an obvious outbreak of respiratory disease had occurred. The calves had not been treated and no death loss was observed in the outbreak. The animals were all killed in a 3-day period and arranged into 3 groups on the basis of the pulmonary findings.

The first group contained 5 calves which had no macroscopic pneumonia and no microscopic evidence of active bronchiolitis. They had, however, a few small peribronchiolar accumulations of cells of the lymphocytic series in some sections of the lungs. These

accumulations were not considered to be significant.

Three calves were assigned to the second group. They had no gross lesions of pneumonia but there was microscopic evidence of a mild bronchiolitis in some sections. There were peribronchiolar lymphocyte accumulations which were sometimes suspiciously large but were not considered to be forming cuffs. These were usually confined to the connective tissue in the peribronchiolar region and the muscularis of the bronchiole was recognizable. Occasional lymphocyte accumulations and scattered neutrophils appeared to be responsible for thickening of the lamina propria.

The remaining calves in the third group had both gross and microscopic pneumonia which were described as characteristic of cuffing pneumonia. These lesions involved large numbers of bronchioles and were accompanied by bronchiolitis and alveolitis. This group in contrast to the first two groups which were considered normal, was considered to be the only one having pneumonia.

A significant association was demonstrated between pneumonic animals and the frequency of mycoplasma isolations. The report indicated that the isolation frequency of M. dispar and Ureaplasma spp. was higher from a pneumonic group of calves than from a group of calves with no microscopic lung lesions. In fact M. dispar was not recovered from any calf in this group. These data are comparable with those of Gourlay et al. (1970) as far as the percentage of calves yielding M. dispar and Ureaplasma spp. is concerned.

The results and observations obtained from the above work (Pirie and Allan, 1975) were combined in a later report which included 56 animals (Allan et al., 1976). The microbiological and pathological observations were expanded and electron microscopy results were included. It was reported that the overall isolation of M. dispar was around 50 percent as previously reported. It was also confirmed that mycoplasmas are found in only a small number of non-pneumonic calves, aged between one and six months, even though this is in contrast to the findings of Thomas and Smith (1972). Very young animals did not yield M. dispar even in the presence of pulmonary lesions. A further observation was that the histopathologic patterns of lung lesions were related to age groups.

Pneumonic lungs of one-month-old calves from which no M. dispar was isolated were characterized by moderate to severe suppurative exudative pneumonia with multifocal abscess formation.

Pneumonic lungs from 2 to 3-month-old calves frequently yielded M. dispar. Histopathology revealed bronchitis, bronchiolitis, and lymphoid accumulations around the small bronchi and bronchioles. These lymphocytic aggregates, however, formed a diffuse layer around the airway with only a few follicular arrangements. The accumulations were present in the submucosal regions and were seen to displace the muscularis and infiltrate the lamina propria. Alveolitis and alveolar collapse were also frequent features and many bronchioles were plugged with mucus and inflammatory exudate.

The lesions found in the pneumonic lungs of the 6-month-old

group were considered typical of cuffing pneumonia. Bronchitis was accompanied by bronchiolitis and follicular lymphoid accumulations ensheathing the bronchioles. The muscularis of many airways was obliterated by infiltrating lymphocytes which extended into the lamina propria. Germinal centers were seen in many of the accumulations. There was also alveolitis with neutrophils, macrophages and occasional plasma cells and giant cells in some cases and alveolar collapse in others. The authors suggested that this classical cuffing pneumonia is the result of the progression from the set of lesions described in the previous paragraph.

Allan et al. (1976) also obtained interesting results concerning electron microscopic examination of pneumonic lungs of their calf series. They found mycoplasmas in five out of eleven pneumonic lungs which were culturally positive for mycoplasmas other than M. dispar. On the other hand, mycoplasmas were demonstrated by electron microscopy in ten out of thirteen pneumonic lungs from which M. dispar was cultured either alone or with other mycoplasmas. It was suggested, then, that the presence of M. dispar significantly increases the probability of detecting mycoplasmas by electron microscopy, as indicated by a Chi-square statistical analysis.

Gourlay et al. (1976b) attempted to discover the cause of cuffing pneumonia in calves produced by the endobronchial inoculation of homogenates of pneumonic lungs. By the use of antibiotic treatment, evidence was obtained that mycoplasmas were responsible for the cuffing pneumonia. The microorganisms most consistently isolated were

M. dispar and ureaplasmas.

Howard et al. (1976) were able to fulfill Koch's postulates in an experiment in which cultures of M. dispar and ureaplasmas were inoculated endobronchially into gnotobiotic calves. Lesions were produced and were estimated to involve 2 to 10 percent of the lung. The organisms were reisolated from the pneumonic lungs, even though no clinical signs could be observed. Therefore, the authors suggested that clinical disease may involve an interaction between other microorganisms and mycoplasmas.

A tentative association of pathological changes and microbiological isolations was reported by Oghiso et al. (1976) and more recently by Schiefer et al. (1978) and Bryson et al. (1978b). The Oghiso and Bryson reports considered both mycoplasmas and other types of agents and the Schiefer's report was restricted to the pattern of lesions seen in P. multocida versus P. hemolytica.

MATERIALS AND METHODS

Experimental Animals

Number, age, source and rearing methods

Twenty-four male, Holstein-Friesian calves were utilized in this study. Difficulties in obtaining all animals at a desirable age range and from the same source, in having available space (isolation units), and in handling too many samples daily indicated that no more than eight calves could be used at one time. Hence, the study was conducted in four different experiments.

Eight calves were utilized in the first experiment. These came from a private farm near Ames, Iowa. They were three to four weeks old when they came from the farm and were assigned to four isolation units (two calves in each unit). They were acclimated there for eight days before actually entering the experiment.

Sixteen calves were used in the remaining three experiments. These all came from the Iowa State University Research Farm, Ankeny, Iowa. They were obtained on the day of calving or on the next day, fed colostrum before movement, and reared together in isolation units. Since this source was able to provide about two calves per week, it usually took at least four weeks to form a group of eight calves which differed up to four weeks in age. An overall evaluation of the animals used in this study shows an age range of one to seven weeks by the time they actually entered the experiment. Most, however, were

approximately four weeks old. They were acclimated for at least 3 days after being assigned to their new isolation units in the randomization process and prior to starting an experiment.

Housing

Two buildings at the Iowa State University Veterinary Medical Research Institute were used for housing the experimental animals. Two units in one building were used for temporary housing and four uniform, air and temperature controlled units in a separate building served for the actual experiment. Each of these last units housed up to two calves at one time in each experiment. Dry straw was used for bedding. The pens were cleaned daily and new straw was supplied.

Feeding

The calves were fed twice daily (7:00 A.M. and 5:30 P.M.) 200g of milk substitute[1] containing 20% crude protein, 20% crude fat, vitamins and minerals. They also had a starter ration[2], grass hay and water ad libitum.

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[1]Formula 308 of Agri-Group Domain Industries, Inc., New Richmond, Wisconsin 54017

[2]Calf-er, of Agri-Group Domain Industries, Inc., New Richmond, Wisconsin 54017.

Additional care and handling

The animals were frequently observed for clinical signs of disease.

Each isolation unit contained the material necessary for cleaning and feeding. Long sleeved coveralls and boots were also kept in each unit for use whenever handling of an animal was needed. Animal caretakers went from non-infected to infected units in the following order: CONTROL, DEXONLY, SOMYCO, MYCODEX (see group definition below).

Experimental Design

Definition of the groups

The final composition within each group as seen in table 1 was obtained after four consecutive experiments had been conducted. Each experiment contributed none, one, or more animals for a particular group. The four groups were as follows:

MYCODEX is a group composed of 7 animals which were treated with one daily dose of 10 mg of dexamethasone[1] intravenously for at least ten consecutive days during which time a single exposure to M. dispar was given.

SOMYCO is defined as that group of 7 animals receiving sterile saline rather than dexamethasone, coincident with the dexamethasone

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[1]Azium, Pfizer, Inc., New York, N.Y.

period of the MYCODEX group. These were exposed to M. dispar as well.

DEXONLY was a group of 5 animals treated with dexamethasone for at least 10 days coincident with that period of the MYCODEX group, but on the day of exposure they were inoculated with sterile mycoplasma medium instead of being exposed to M. dispar containing medium.

CONTROL is the group of 5 animals that were given daily injections of sterile saline for at least ten days and inoculated with sterile mycoplasma medium.

Description of the experiments

Experiment 1 This experiment was conducted with the first eight calves that came from a private farm. Two animals were used for each group. Each animal in the MYCODEX and in the DEXONLY groups was given 5 ml of a solution containing 2 mg of dexamethasone per ml intravenously every morning from day minus four (-4) through day five (10 days). Each animal in the SOMYCO and CONTROL groups was given 5 ml of sterile saline intravenously every morning from day minus four (-4) to day five. Each animal of the MYCODEX and SOMYCO groups was intratracheally inoculated with 10 ml of broth medium containing 10^9 colony-forming units of M. dispar per ml at day zero. Each animal in the DEXONLY and CONTROL groups was given 10 ml of sterile mycoplasma medium by the same route. The inoculations in this experiment were performed using a 16 gauge, 2.5 inch needle and a 10 ml plastic syringe. One animal from each group in this experiment was killed at day 12 and one at day 16 post inoculation (PI).

Table 1. Distribution of experimental animals among treatment groups in four experiments

Group	MYCODEX[a]			SOMYCO[b]			DEXONLY[c]			CONTROL[d]			
Days PI[e]	12	16	20	12	16	20	12	16	20	12	16	20	Total
Experiment													
1st	7103[f]	7600		7105	7104		7102	7109		7599	7108		8
2nd	57		31	37		98	24[g]		14	17		12	8
3rd			102			101							2
4th		72	75[h]		74	76[h]			73[h]			77[h]	6
Total	2	2	3	2	2	3	2	1	2	2	1	2	24
Overall		7			7			5			5		24

[a]MYCODEX = calves given dexamethasone and exposed to M. dispar.
[b]SOMYCO = calves given saline and exposed to M. dispar.
[c]DEXONLY = calves given dexamethasone and sterile mycoplasma medium.
[d]CONTROL = calves given saline and sterile mycoplasma medium.
[e]numbers refer to day of scheduled necropsy (PI = post inoculation).
[f]calf was killed on day 11 post inoculation because he was in extremis.
[g]calf died on day 11 post inoculation.
[h]Necropsied on day 21 PI.

Experiment 2 Eight calves from the Iowa State University Research Farm were used in experiment 2. The calves were distributed among groups (two animals in each group) and treated similarly to those of experiment 1 except that one animal of each group was necropsied at day 12 and one at day 20 PI.

Experiment 3 Two animals from the Iowa State University Research Farm were used in experiment 3. One was in the MYCODEX and one in the SOMYCO group and as such they were treated the same as calves from the previous experiment in the analogous groups, except that the amount of inoculum was doubled. Both calves were necropsied at day 20 PI.

Experiment 4 Six calves from the Iowa State University Research Farm were used in experiment 4. Two of them were assigned to the group MYCODEX, two to SOMYCO, one to DEXONLY and one to CONTROL. Each animal in the MYCODEX and DEXONLY groups was given 5 ml of a solution containing 2 mg of dexamethasone per ml intravenously every morning from day minus seven (-7) to day four (12 days). Each animal in SOMYCO and CONTROL groups was given 5 ml of sterile saline intravenously every morning from day minus seven (-7) to day four (12 days). Each animal of the MYCODEX and SOMYCO groups was endobronchially inoculated with 20 ml of medium containing 10^9 colony-forming units per ml of M. dispar at day zero of that series and each one in the DEXONLY and CONTROL groups was given a 20 ml amount of sterile mycoplasma medium. The endobronchial inoculation in this experiment was performed as

follows: the calves were locally anesthetized with 5 to 10 ml of lidocaine hydrochloride[1] injected subcutaneously in the ventral anterior cervical region. A small, longitudinal incision was made at this site, the trachea exposed and a 6 mm trocar inserted between tracheal rings. A sterile plastic catheter which was three mm in diameter was passed through the trocar into the trachea and down into the lower respiratory tract to impact in a bronchiole. Ten ml of sterile broth were passed down the tubing by means of a syringe. A one ml portion of material was aspirated back for use in microbiological examinations (pre-inoculation). A second syringe containing 20 ml of broth culture with 10^9 colony-forming units per ml was then attached to the tubing and the material dispensed into the lower respiratory tract, followed by 20 ml of air. The tubing and trocar were withdrawn and the skin incision closed with nylon sutures. The animals in groups DEXONLY and CONTROL were also inoculated on day zero with sterile mycoplasma medium in the same manner and amounts as was used for their partners from a particular experiment. One calf from each of MYCODEX and SOMYCO groups was necropsied at day 16 and the rest were necropsied at day 21 PI.

Experiments 1, 2 and 3 were conducted in the Summer of 1978 and experiment 4 early in the following Fall.

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[1] Xylocaine(R), Astra, Worcester, Mass. 01606.

Parameters Evaluated

Two sets of data were considered in this study: (a) those obtained from the day an animal entered the experiment to the date of necropsy (Table 2) and (b) those obtained upon or after a necropsy had been performed.

Rectal temperature and respiratory rate

The rectal temperature was taken twice daily before feeding. The respiration was counted twice daily at two to three hours after feeding and handling when the calves were usually found to be lying down or at least resting very quietly.

Hematologic parameters

Each morning a 5 ml sample of blood was drawn from the jugular vein of each calf for hematology, using an ethylenediaminetetraacetic acid (EDTA) containing vacutainer tube[1]. The blood samples were obtained between 7:00 and 8:00 A.M. before feeding and dexamethasone or saline injections. Packed cell volume (PCV), hemoglobin concentration (Hb), total white blood cell count (WBC), total plasma protein (PP) and fibrinogen level were determined soon after collection of samples. A thin blood smear for each blood sample was also made and stained by the Wright's stain technique for differential white blood cell counts. The techniques used in each case are as follows: PCV was

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[1]Becton-Dickinson and Co., Rutherford, New Jersey.

Table 2. Parameters measured in all animals during the course of the experiment

Parameters	Days pre or post inoculation with *Mycoplasma dispar* or sterile medium																							
	-4[a]	-3	-2	-1	0[b]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20[c]
Rectal temperature (2 times a day)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiration rate (2 times a day)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PCV[d]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hb[e]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WBC[f] count	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
WBC differential	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Plasma protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Plasma fibrinogen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Culturing for *M. dispar* (nasal swabs)	+		+		+		+		+		+		+		+		+		+		+		+		+

[a]Observations started on day -7 in six animals (experiment 4).

[b]Inoculation with *Mycoplasma dispar* (infected) or sterile medium (uninfected).

[c]All observations were recorded up to the day calf was killed (11, 12, 16, 20, 21).

[d]PCV = packed cell volume.

[e]Hb = hemoglobin.

[f]WBC = white blood cell.

determined by the microhematocrit technique[1], fibrinogen and PP were determined by using a T/S refractometer[2] and total WBC counts were obtained with an electronic cell counter[3]. The percentage of each cell type was obtained by microscopic counting of a total of 100 cells under oil immersion and the absolute number was calculated from those figures.

Microbiological procedures

A nasal swab was obtained every second morning starting on the first day of the experiment and continuing to the day of necropsy. Hence, at least three pre-inoculation samples were used to attempt mycoplasma isolation.

A variety of materials were also collected from the animals at necropsy. Nasopharynx, upper trachea and lower trachea were regularly swabbed. At least one piece of the right cranial lobe of the lung and pieces of bronchial and mediastinal lymph nodes, pharynx and tonsils were collected for microbiology. In the few instances when lesions were more diffuse or when they were mostly seen in areas other than the right cranial lobe, additional areas were also sampled to include representative lung lesions. Occasionally other mucosal surfaces were swabbed and other fluids and tissues collected for the same purposes.

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[1]Clay Adams Autocrit Centrifuge, Division of Becton-Dickinson and Co., Parsippany, New Jersey 07054.

[2]American Optical Company, Scientific Instrument Division, P. O. Box 66499, Chicago, Ill. 60666.

[3]Coulter Counter ZBI, Coulter Electronics, Inc., 590 W. 29th St., Hialeah, Florida 33010.

The culture media (both broth and solid) for mycoplasma isolations were those recommended for Mycoplasma hyopneumoniae and Mycoplasma flocculare (Friis, 1975) and later adapted for isolation of bovine mycoplasmas (Bitsch et al., 1976). The composition and directions for preparing the above and several other mycoplasma media have been compiled elsewhere (Stalheim, 1976).

Nasal swabs or swabs from post-mortem materials were placed into 1.9 ml of Friis broth and allowed to equilibrate for at least 1 hour. The absorbed medium was expressed from the swab. Two tenths ml quantities of the absorbed media were dispensed into the first of a series of 10 tubes, each containing 1.8 ml of broth. Subsequently, 0.2 ml was transfered from tube to tube to obtain a 10-fold dilution series up to 10^{-10}.

Tissues were processed by homogenizing approximately 1 g of lung, pharynx, bronchial lymph node, mediastinal lymph node, trachea or occasionally other tissues in a Ten-Broeck grinder and suspending in Friis broth. Serial ten-fold dilutions of these suspensions were prepared by delivering 0.2 ml of the 1:10 suspension to 1.8 ml broth and then by serially diluting to 10^{-10}.

The inoculated broths were incubated at $37^{o}C$ in 5 percent CO_2 and observed daily for a shift in pH as indicated by medium changing from pink to yellow. Turbidity was also used as an indicator of growth. A loop of the culture suspected of containing mycoplasmas was streaked onto Friis agar and incubated at $37^{o}C$ in 5 percent CO_2

and observed daily for growth by scanning the agar under an inverted microscope.

Modified Hayflick's medium (Livingston and Gauer, 1976) was used to attempt ureaplasma isolation. The procedures followed were the same as those used for mycoplasma isolation.

A bacteriological loop was used to obtain bronchial secretions or exudate (when present) from pieces of lung. The material was streaked onto plates, incubated and examined as above.

Tissue homogenates and bronchial secretions or exudate were also streaked onto 5 percent sheep blood agar and incubated as above for observing any bacterial growth. Nurse staphylococcal colonies were used to detect hemophilus growth.

Growth inhibition and growth precipitation tests were used to identify the mycoplasma isolants. Horse hyperimmune antisera against M. dispar, M. bovis and M. bovirhinis were used for identification purposes[1].

Fluorescent antibody technique (FAT)

Tissues routinely collected for immunofluorescence were cranial lobe of the right lung, lower trachea, upper trachea, nasopharynx and tonsil. Other tissues which also were sampled in most of the animals

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[1]All horse antisera to mycoplasmas were obtained from Dr. O. H. V. Stalheim, National Animal Disease Center, Ames, Iowa 50010.

included bronchial and mediastinal lymph nodes, kidney, bladder, urethra and spleen.

Tissues were sectioned at 8 µm with a cryostat microtome[1] for indirect immunofluorescence (FAT) staining. Several sections of each tissue were obtained. They were fixed with cold acetone for 5 minutes. Individual sections were covered with horse antisera to M. dispar, M. bovis, M. bovirhinis and M. bovoculi[2]. The slides were incubated for 90 minutes at 37°C. The sections were rinsed 2 times for 5 minutes each time in a phosphate buffered saline solution (pH 7.2) and stained with fluorescein conjugated[3] rabbit anti-equine gamma-globulin for 30 minutes at 37°C. The sections were again rinsed 2 times for 5 minutes each time in the phosphate buffered saline solution (pH 7.2) and then 2 two-minute rinses in distilled water. The slides were mounted in pH 8.6 buffered glycerin and examined under a microscope supplied with ultraviolet light. Exudate or normal bronchial secretion was expressed or swabbed from a bronchus and was smeared on a clean slide for FAT staining as described for tissues.

Necropsy

Except for calf number 24 which died prior to the scheduled day, all calves were electrocuted, including calf 7103 which was

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[1] Cryo-cut microtome, American Optical Corporation, Scientific Instrument Division, Buffalo, N. Y. 14215.

[2] All horse antisera to mycoplasmas were obtained from Dr. O. H. V. Stalheim, National Animal Disease Center, Ames, Iowa 50010.

[3] Cappl Laboratory, Inc., Downingtown, PA.

necropsied 1 day prior to the scheduled day because he was in extremis. The calves were immediately laid on their left side for necropsy. A ventral mid-longitudinal incision was made, the skin reflected, and superficial lymph nodes were obtained for histopathology. The right rib cage was removed to allow inspection of the thoracic cavity and a visual evaluation of thoracic organs. As the right lung was exposed, the pleural surface and the pericardial sac were swabbed for microbiological examination in some of the animals. The whole respiratory tract was then removed together with the heart, thymus, esophagus and tongue. These tissues were put on a clean table and the visual evaluation completed. Any abnormalities noted in the lungs were recorded in drawings and were described. Photographs were made from a few cases. Materials for microbiology and fluorescent antibody techniques were taken as described previously.

Histopathology

Harvesting and fixation procedures for lung tissue differed slightly in each experiment. In experiments 1 and 2 a small piece of tissue was taken from the edge of each of 10 different areas throughout the lung and was harvested from wherever a lesion was noticed. The tissues were put in properly identified bottles which contained 10% buffered formalin. In experiment 3, pieces of tissues were taken as above from the first 6 monitored areas (see below) of the right lobes but the left lung was fixed by endobronchial perfusion before obtaining the small pieces from areas 7 through 10. In experiment 4 the tissues for

microbiology and immunofluorescence were taken from the right cranial lobe and the rest of the lung was perfused endobronchially. The endobronchial perfusion in both experiments 3 and 4 was performed by dripping 10% buffered formalin into the bronchial tree. The lower trachea or the sectioned bronchus was tied in order to keep the fixative in, and the tissues were immersed in a 10% buffered formalin bath. The lungs and the other tissues were allowed to be in this formalin bath for at least 48 hours for complete fixation before cutting. The same 10 chosen areas of each lung yielded properly identified sections for adequate monitoring. Several cross sections of the lung lobes also provided materials which allowed evaluation of major bronchi at different levels. Tissues harvested for histopathology also included turbinate, tonsil, pharynx and trachea. Small pieces of the above tissues were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin for histopathology. Lung lesions were ranked on the basis of observations of many lung sections. Figure 1 is a drawing of a calf lung. Its purpose is to indicate 10 different areas throughout the lung lobes where tissues were taken. Each of these areas supplied a minimum of 2 sections, one of which was perpendicular and the other transverse to the lobe edge. Other nonidentified areas were also taken, especially with the purpose of having main bronchi examined. These usually came from cross sections of different segments of the lung lobes. A zero to four system was instituted for ranking the lung lesions. The system was based upon careful observation of 5 particular

Figure 1: Schematic drawing of a calf lung showing tissue sample collection sites

A = Cranial pars of the right cranial lobe

B = Caudal pars of the right cranial lobe

C = Middle lobe

D = Right caudal lobe

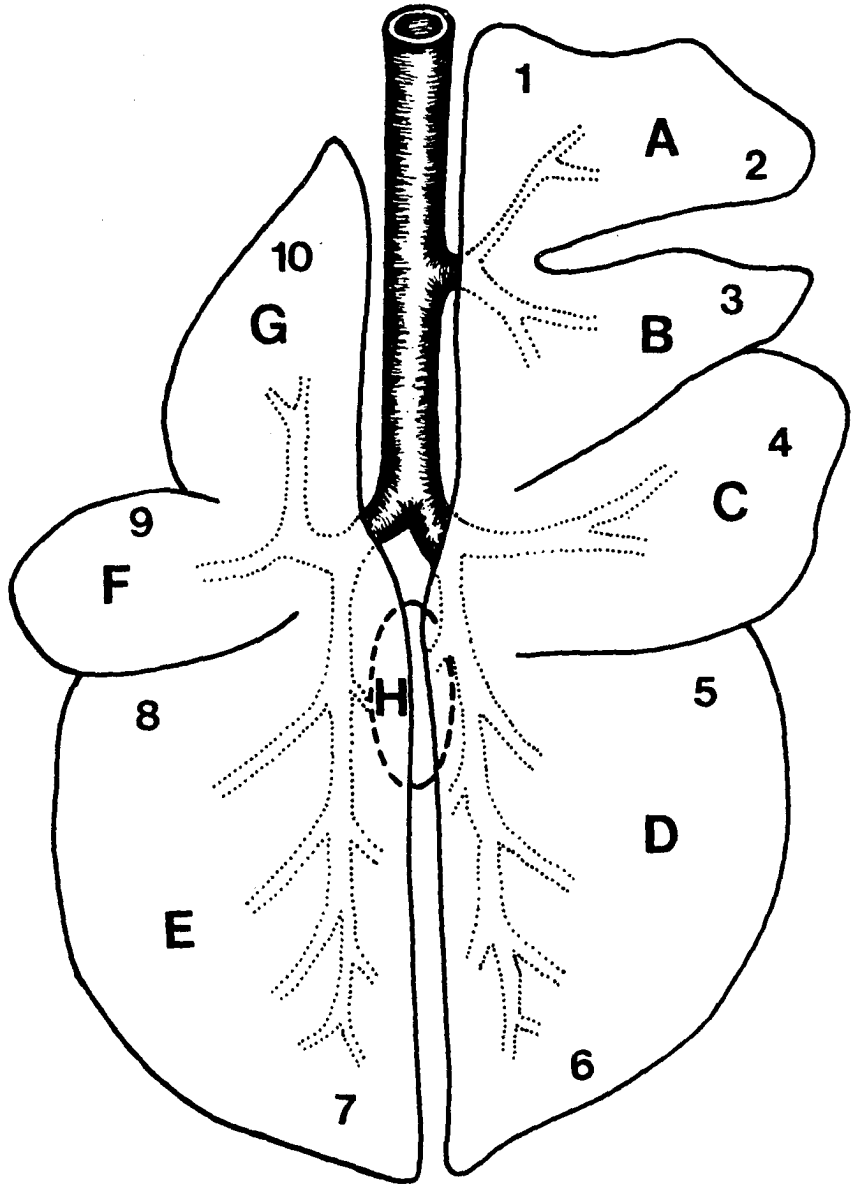
E = Left caudal lobe

F = Caudal pars of the left cranial lobe

G = Cranial pars of the left cranial lobe

H = Accessory lobe

Numbers 1 to 10 indicate sampling areas


1
A
2
10
G
B
3
4
C
9
F
H
5
8
D
E
6
7

types of lesions: (1) peribronchial and peribronchiolar lymphoid hyperplasia, (2) bronchiolitis, (3) interstitial thickening, (4) alveolitis and (5) granulomatous lesions. Each lesion classification was evaluated in all sections before going on to the next type of lesion.

The amount of lymphoid tissue, its aggregation pattern, the integrity of the basement membrane, and the extent of lymphocytic invasion into the lamina propria were the criteria used to score the peribronchial and peribronchiolar lymphoid hyperplasia. The number of lymphocytes around blood vessels was the criterion to ascertain perivascular lymphoid accumulation and this usually helped to evaluate the lymphocytic reaction.

Bronchitis and bronchiolitis were evaluated on the basis of the nature and extent of inflammatory exudate in the lumen, the extent of epithelial and ciliary damage, and on the basis of neutrophil infiltration in the mucosa, lamina propria and surrounding tissue. Bronchial epithelial hyperplasia was also included.

The interstitial thickening was scored on the basis of reduplication of cell layers and cellular infiltrate in the alveolar walls.

Criteria for scoring alveolitis were the presence and extent of inflammatory infiltrate in alveolar spaces.

The granulomatous lesions were scored only on the basis of number of granulomas per section, since they had similar histological appearance and were all of minute size.

The ranking system represents a summary of the whole extent of the evaluation of a lung as it compared with observations of the lungs of all other calves. The granulomatous lesions, however, were not considered for the overall score given to each lung.

A few selected slides were used for special stains.

A minimum of two sections representing upper and lower trachea were evaluated histologically. A zero (normal) to four (most severe) scoring system was instituted to represent the degree of tracheal lesions. The lamina propria was examined for the extent of mononuclear and neutrophil infiltrations and for its blood supply. The mucosa was examined for epithelial vacuolation, neutrophil and mononuclear infiltrations, the relative number of goblet cells, clumping and loss of cilia, erosions and superficial deposition of exudate. A single score was then arrived at as a relative grade based on the combined observations. The same kind of evaluation was applied to one section of the nasal turbinate from each calf.

Pharynxes and tonsils were taken from most of the calves for both fluorescent antibody and histopathological examinations. It was later realized that inconsistencies in regard to the site of tissue harvesting resulted in evaluation of oropharyngeal and nasopharyngeal mucosae, tonsilar crypts and sinuses, palatine tonsils, and nasopharyngeal tonsils of only 11 calves. These were considered a representative sample of the pharyngeal tissue which could be evaluated through a scoring system which was based on the extent of inflammatory exudate and debris in the

tonsilar crypts and sinuses, vacuolation of epithelial cells, neutrophil infiltration in the oropharyngeal and nasopharyngeal mucosa and lamina propria and changes in the cilia of nasopharyngeal epithelium. One or more of these tissues were not collected from the other calves. The available tissues were evaluated but no scoring was attempted.

Electron microscopy

Electron microscopy was utilized to evaluate ultrastructural characteristics of selected tissues. Fresh tissues or, in some cases, formalin fixed tissues which had been rinsed with buffer were cut into approximately 1 mm squares and placed in ruthenium red-glutaraldehyde fixative. The fixative contained 4% glutaraldehyde, 0.1 M cacodylate buffer at pH 6.5, and 1.5 mg per ml of ruthenium red (Howard and Gourlay, 1974).

After overnight fixation, tissues were rinsed in 0.1 M cacodylate buffer and post-fixed for 2 hours at room temperature in osmium tetroxide-ruthenium red post-fixative. Composition included 4% osmium tetroxide, 0.1 M cacodylate buffer at pH 6.5, and 1.5 mg per ml of ruthenium red. Dehydration in a graded ethanol series was followed by embedding in Epon 812 mixture which contained 45.7 ml Epon 812, 21.0 ml dodecenylsuccinic anhydride (DDSA), 23.6 ml nadic methyl anhydride (NMA), and 1.5 ml, 2,4,4-tri(dimethyl amino methyl) phenol (DMP 30)[1].

One to two μm sections were cut for block orientation and were stained with toluidine blue stain. Thin sections were cut with an

[1]Epon 812, DDSA, NMA and DMP 30, Electron Microscopy Sciences, Box 251, Fort Washington, PA 19034.

LKB[1] microtome and stained in 2% methanolic uranyl acetate and Reynolds lead citrate (Reynolds, 1963) prior to observation in a Hitachi HS-9[2] electron microscope.

Analysis of variance plan

An average for each variable measured daily for each calf was obtained. This average was considered as a single datum for each calf. The set of data supplied by the twenty-four calves (twenty-three degrees of freedom), which were distributed among four different groups (three degrees of freedom) and the lung lesion scores were analyzed in a two way analysis of variance. Therefore, there were twenty degrees of freedom for error. The three degrees of freedom from the model were separately analyzed to obtain the contribution supplied by the dexamethasone effect (one degree of freedom), by the infection effect (one degree of freedom), and by the interaction between dexamethasone and infection effects (one degree of freedom). Data analyzed were morning respiration, evening respiration, morning temperature, evening temperature, total white blood cell count, absolute numbers of neutrophils, absolute numbers of lymphocytes and plasma protein:fibrinogen ratio.

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[1]LKB ultratome 1, LKB producer AB, Rockville, MD 20850.

[2]Hitachi Electron Microscope, Hitachi Perkin-Elmer, Chicago, Illinois 60607.

RESULTS

Clinical Signs

Diarrhea was frequently observed in dexamethasone-treated animals. It started near day zero (5th day of dexamethasone injections), was severe for the rest of the dexamethasone treatment period and slowly decreased in severity during the next four to six days. However, in two calves (calf 7103 of MYCODEX/experiment 1 and calf 24 of DEXONLY/experiment 2) a severe diarrhea persisted and contributed to the death of the animals which occurred on day 11 PI.

Several calves had alopecia involving mainly the face, flank and hind quarters. The specific causes could not be determined.

All animals had a transient cough soon after being given the intratracheal inoculum (sterile or containing M. dispar). The majority of the animals in experiment 4 which were inoculated endobronchially through a surgical procedure had mild but more prolonged coughing which was mainly observed in calves 72 and 75 of the MYCODEX group and calf 74 of the SOMYCO group. However, most of the animals throughout all experiments were essentially free of respiratory clinical signs. Calf 7600 (MYCODEX/experiment 1) was the only animal which had a clinical pneumonia consisting of persistent non-productive cough and dyspnea which started on day 5 PI and continued up to day 16 PI when the calf was killed and necropsied. This calf had several days of high rectal temperature (39.9 to 41^{o}C). He also had an increased respiratory rate with deep and costo-abdominal movements and with audible rales, and became lethargic and anorectic. There was

leucocytosis with neutrophilia after a few days of recession from the dexamethasone effect. Calf 7600 also had a markedly decreased plasma protein:fibrinogen ratio.

Microbiology

Isolation of M. dispar from nasal swabs of live experimental animals

The pattern of upper respiratory shedding of M. dispar is indicated in Table 3.

Animals in experiment 1 began to shed M. dispar between days 6 and 10 PI. The organism was first detected at day 10 PI in calves 7103 and 7600 both of the MYCODEX group. Calf 7103 died on day 11 PI so that the organism was detected only one time during the experimental period.

Calves 7104 and 7105 of the SOMYCO group began to shed the organism at day 6 PI.

Calves 7102 and 7109 of the DEXONLY group, and 7108 and 7599 of the CONTROL group were not intentionally infected but also began to shed the organism at about the same time (6 to 10 days PI). So, further references to infected calves will include these 4 animals.

No M. dispar could be detected in nasal swabs of any of the eight calves of experiment 2 (calves 12, 14, 17, 24, 31, 37, 57 and 98).

Calf 101 of the SOMYCO group and calf 102 of the MYCODEX group (both from experiment 3) had the most consistent pattern of M. dispar shedding among all experiments. It was first detected on day 2 PI and continued for the rest of the experimental period. Calf 102 had

Table 3. Pattern of nasal shedding of *Mycoplasma dispar* during pre- and post inoculation period

Group	Calf No.	Days pre or post inoculation												
		-4	-2	0	2	4	6	8	10	12	14	16	18	20
MYCODEX	7103	-[a]	-	-	-	-	-	-	3[b]					
	57	-	-	-	-	-	-	-	-	-				
	7600	-	-	-	-	-	-	-	2	3	3	4		
	72	-	-	-	2	-	-	-	-	-	-	-		
	31	-	-	-	-	-	-	-	-	-	-	-	-	-
	102	-	-	-	1	2	-	1	1	2	1	2	2	2
	75	-	-	-	-	-	-	-	-	-	-	-	-	-
SOMYCO	7105	-	-	-	-	-	1	1	2	4				
	37	-	-	-	-	-	-	-	-	-				
	7104	-	-	-	-	-	2	1	-	3	2	3		
	74	-	-	-	-	-	-	-	-	-	-	-		
	98	-	-	-	-	-	-	-	-	-	-	-	-	-
	101	-	-	-	2	3	4	4	5	6	6	6	6	5
	76	-	-	-	-	-	-	-	-	-	-	-	-	-
DEXONLY	7102	-	-	-	-	-	2	1	2	3				
	24	-	-	-	-	-	-	-	-					
	7109	-	-	-	-	-	-	-	3	5	5	4		
	14	-	-	-	-	-	-	-	-	-	-	-	-	-
	73	-	-	-	-	-	-	-	-	-	-	-	-	-
CONTROL	7599	-	-	-	-	-	1	1	2	1				
	17	-	-	-	-	-	-	-	-	-				
	7108	-	-	-	-	-	-	1	-	2	4	4		
	12	-	-	-	-	-	-	-	-	-	-	-	-	-
	77	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]Indicates negative *M. dispar* isolation results.

[b]Figures indicate reciprocal of the $\log_{10}$ of *M. dispar* titers.

low titers (10^1 or 10^2) and calf 101 had increasing titers of 10^2 up to 10^6 throughout the experimental period.

Calf 72 (MYCODEX/experiment 4) yielded 10^2 M. dispar from the swab taken at day 2. However, M. dispar could not be isolated from previous or subsequent nasal swabs of this calf. Nasal swabs taken from the other calves in experiment 4 (73, 74, 75, 76 and 77) were all negative for M. dispar.

Isolation of M. dispar, bacteria and fungi from post mortem materials

M. dispar was recovered from a single or from multiple sites of the respiratory tract of all 11 calves which shed the organisms in life (Table 3) and from five other calves from which nasal swabs yielded no mycoplasma. The titers and specific sites of M. dispar isolations in these 16 infected calves are recorded in table 4. The lungs usually yielded M. dispar titers as high as 10^4 to 10^6. Trachea, pharynx and nasal cavity titers were high and paralleled the high titers observed in the lung.

Twelve of the above sixteen infected calves had M. dispar titers of 10^4 or above in either the trachea, the pharynx, or in both. Calf 7600 (MYCODEX/experiment 1) yielded titers as high as 10^7 from both trachea and pharynx. In addition, M. dispar was isolated from the tonsil and bronchial lymph node of calf 74 (SOMYCO/experiment 4).

Urine, kidney, spleen, mediastinal lymph node, pericardial fluid and joint fluid were all negative on culture for either bacteria or M. dispar. Swabs from pericardial surface, joint surface and conjunctival sac also gave negative results for M. dispar or bacteria.

Table 4. Evidence of *Mycoplasma dispar* involvement in the respiratory tract of experimentally infected calves

Group	Calf No.	Lesion Score				Electron Microscopy			Fluorescent Antibody Technique				
		Lu[a]	Tr	Ph	Tu	Lu	Tr	Ph	Lu	BS	Lt	Ut	Ph
MYCODEX	7103	1	1	ND[b]	0	-	ND	ND	+	+	ND	ND	-
	57	1	0	1	1	ND	-	ND	-	-	ND	ND	-
	7600	3	4	ND	4	+	ND	ND	+	+	ND	ND	+
	72	2	2	ND	1	-	+	+	-	-	-	-	+
	31	1	0	ND	0	ND	ND	ND	-	-	ND	ND	-
	102	1	0	ND	1	ND	ND	ND	-	-	ND	ND	+
	75	1	2	0	2	ND	ND	+	+	-	+	-	+
SOMYCO	7105	1	0	ND	0	-	ND	ND	+	+	ND	ND	ND
	37	1	0	0	0	ND	ND	ND	-	-	-	-	-
	7104	1	3	ND	3	ND	ND	ND	+	+	ND	ND	+
	74	1	1	1	2	ND	+	-	-	-	-	-	+
	98	1	0	2	1	ND	ND	ND	-	-	ND	ND	-
	101	1	0	1	1	ND	ND	ND	-	-	ND	ND	-
	76	1	0	1	1	-	+	+	-	-	+	+	+
DEXONLY	7102	1	3	ND	2	-	ND	ND	+	-	ND	ND	ND
	24	0	0	ND	0	ND	ND	ND	-	-	ND	ND	-
	7109	1	2	ND	4	-	ND	ND	+	+	ND	ND	+
	14	1	1	ND	1	ND	ND	ND	-	-	ND	ND	-
	73	0	0	0	1	ND	-	-	-	-	-	-	-
CONTROL	7599	0	3	ND	2	ND	ND	ND	+	+	ND	ND	ND
	17	1	0	0	0	ND	ND	ND	-	-	ND	ND	-
	7108	0	3	ND	1	+	ND	ND	+	+	ND	ND	+
	12	0	0	0	0	ND	ND	ND	-	-	ND	ND	-
	77	1	0	0	0	ND	-	ND	-	-	-	-	-

[a]Letters are abbreviations indicating the following specific tissue sites: Lu = lung, Tr = trachea, Ph = pharynx, Tu = turbinate, BS = bronchial swab, Lt = lower trachea, Ut = upper trachea, Ts = turbinate swab.

[b]Not done.

[c]Indicates $\log_{10}$ *M. dispar* recovered from swabs or 1 g of tissue.

[d]Indicates *M. dispar* could not be isolated.

M. dispar titer					Lung yields of organisms other than *M. dispar*
Lu	BS	Tr	Ph	Ts	
5[c]	4[d]	5	ND	4	α-*Streptococcus*
1	-	-	-	-	
6	6	7	7	4	*Pseudomonas* sp., *Streptomyces*
-	-	5	4	-	
-	-	-	-	-	*Aspergillus niger*, *Aspergillus fumigatus*
-	-	1	5	2	
-	4	3	4	-	*Pseudomonas* sp., *Aspergillus glaucus*
5	6	5	ND	4	*Micrococcus* sp.
1	1	-	-	-	
6	5	-	6	3	
-	-	5	4	-	
1	-	-	-	-	
-	6	2	6	5	
-	-	-	-	-	α-*Streptococcus*, n.h. *Staphylococcus*
6	6	6	ND	4	
-	-	-	-	-	*A. fumigatus*, *A. niger*, α-*Streptococcus*, *Streptomyces*
5	5	5	6	5	*Pseudomonas* sp.
-	-	-	-	-	
-	-	-	-	-	*A. fumigatus*
4	4	1	ND	1	α-*Streptococcus*
-	-	-	-	-	
6	6	6	5	3	*A. fumigatus*
-	-	-	-	-	
-	-	-	-	-	*A. glaucus*

Lung tissue of several calves yielded a few species of bacteria as indicated in table 4. Fungi, also as specified in table 4, were present in the lung tissue of several calves.

Fluorescent Antibody Techniques

The indirect fluorescent antibody test (FAT) was applied to the lungs of all calves, to a smear made of bronchial exudate (or secretion) of all calves, to pharyngeal tissue of 21 of them, and to sections of both lower trachea and upper trachea of all calves of experiment 4 (72, 73, 74, 75, 76 and 77) and of calf 37 (SOMYCO) of experiment 2.

The FAT results are shown in table 4. Positive results for only a single site were observed in four cases. Calf 72 (MYCODEX/experiment 4), calf 74 (SOMYCO/experiment 4) and calf 102 (MYCODEX/experiment 3) had a positive test for the pharyngeal tissue but not for the other sites examined. The lung of calf 7102 (DEXONLY/experiment 1) was FAT positive for M. dispar but the smear of the bronchial exudate was negative.

The overall results indicate that 12 of the 16 calves which yielded M. dispar on culture also had evidence of infection based on FAT. In addition, the trachea and pharynx of calf 76 (SOMYCO/ experiment 4) gave positive FAT results. Since mycoplasma could also be demonstrated in those two tissues by electron microscopy, as will be described under the next heading, calf 76 was considered infected.

The results of FAT were negative for tissues other than the respiratory tract in all animals. Tissues examined were urethra, tonsil, kidney, spleen, bladder and mediastinal lymph nodes.

Electron Microscopy

Electron microscopic examinations were performed on the nasopharynx of calves 72, 74, 75 and 76, and the trachea of calves 72, 74 and 76 which all had M. dispar in the upper respiratory tract as indicated by FAT or microbiology.

The tracheas of calves 57, 73 and 77 and pharynx of calf 73 served as negative controls because M. dispar could not be demonstrated in their upper respiratory tracts by either FAT or culture.

Portions of the lungs from infected calves (72, 76, 7102, 7103, 7105, 7108, 7109 and 7600) were also examined by electron microscopy. Electron microscopic results are recorded in table 4.

Mycoplasma was found by electron microscopy in three of the four pharynxes which were positive by other techniques and mycoplasma was found in all positive tracheas. It was also found in two of the six lungs.

Mycoplasma was not seen in tissues which were M. dispar negative by FAT and culture.

The organisms were in most instances associated with cilia (figures 2, 3 and 4). A delicate but dense material was observed radiating from the cell membrane of the organisms and appeared to be responsible for the close relationship between the organisms and the cilia (Figure 2). Occasional ballooning and branching of the cilia were observed. When clusters of a few organisms were close together the associated cell was rounded and apparently smaller in size. These cells also had larger profiles of secretory granules.

Figure 2. Electron photomicrograph of a ciliated cell from nasopharyngeal mucosa of a Mycoplasma dispar infected calf (75) Notice electron-dense capsular material bridging between the mycoplasma body and adjacent cilia (arrows)

Figure 3. Mycoplasmas associated with the tracheal epithelium of a *Mycoplasma dispar* infected calf (74) - electron photomicrograph Several mycoplasma bodies are seen among cilia and microvilli (arrows)

Figure 4. Clusters of mycoplasmas in the tracheal mucosa of a Mycoplasma dispar infected calf (74) - electron photomicrograph Notice association with a goblet cell (1) and with a ciliated cell (2)


1
2
c

Table 5 illustrates the infection status of calves with M. dispar as based on the results of microbiological isolations, FAT and electron microscopy examinations. The results indicate that 17 calves were infected and 7 were non-infected. Eight of the infected calves were treated with dexamethasone and nine were given saline. Four of the non-infected were given dexamethasone and 3 received saline.

Some of the results to be reported and discussed hereafter will be based on the experimentally demonstrated infective status in order to evaluate effects of infection.

A summarization of all data for eight different parameters which had a treatment effect are presented in tables 6, 7 and 8. The parameters are morning respiration, evening respiration, morning temperature, evening temperature, plasma protein:fibrinogen ratio (PP:F), total white blood cell count, neutrophil count and lymphocyte count.

The overall means in the above tables may not indicate temporary changes caused by dexamethasone (as will be described later) because of the long term measurement. However, changes in the total WBC counts, neutrophil counts, lymphocyte counts and PP:F were sufficiently marked to be detected when overall means were analyzed statistically.

Respiration Rate and Temperature

The dexamethasone treated animals had respiration rates and rectal temperatures (tables 6 and 7) which were slightly higher than saline-treated animals throughout the experiment. The differences in respiration were more pronounced between days 7 and 18 PI. Peaks were

Table 5. Infective status of calves within the dexamethasone and saline treated groups

Infective status	Dexamethasone treated calves	Saline treated calves	Day PI[a] calf was necropsied
infected[c]	7103		11
	57	37	12
	7102[b]	7105	12
		7599[b]	12
	72	74	16
	7109[b]	7104	16
	7600	7108[b]	16
		98	20
	102	101	20
	75	76	21
non-infected[d]	24		11
		17	12
	14		20
	31[e]	12	20
	73	77	21

[a]Post inoculation.

[b]Calves were not intentionally exposed to Mycoplasma dispar but became infected as demonstrated by culture and fluorescent antibody test.

[c]Mycoplasma dispar demonstrated by one or more laboratory means.

[d]No M. dispar could be demonstrated in life or at necropsy.

[e]Calf was exposed to M. dispar but did not become infected.

Table 6. Overall means for eight variables which were measured daily for the 4 different treatments

	Infected dexamethasone	Infected saline	Non-infected dexamethasone	Non-infected saline
MR[a]	39.05	36.68	47.62	41.07
ER[b]	39.85	38.22	49.97	41.59
MT[c]	39.30	39.10	39.33	39.05
ET[d]	39.46	39.25	39.29	39.13
PP:F[e]	8.40	13.17	9.82	13.62
WBC[f]	16200	8733	15032	9788
AN[g]	12092	3402	11447	4147
AL[h]	3396	5062	3174	5319

[a]Morning respiration (numbers of respiration/minute).

[b]Evening respiration (numbers of respiration/minute).

[c]Morning rectal temperature (^{o}C).

[d]Evening rectal temperature (^{o}C).

[e]Plasma protein:fibrinogen ratio.

[f]Total number of white blood cells (per mm^3).

[g]Absolute numbers of neutrophils (per mm^3).

[h]Absolute numbers of lymphocytes (per mm^3).

Table 7. Overall means for eight variables which were measured daily for dexamethasone and saline treated calves

	Dexamethasone	Saline
MR[a]	42.11	37.85
ER[b]	43.49	39.12
MT[c]	39.31	39.09
ET[d]	39.40	39.22
PP:F[e]	5.80	13.29
WBC[f]	15779	9014
AN[g]	11858	3601
AL[h]	3316	5130

[a]Morning respiration (numbers of respiration/minute).

[b]Evening respiration (numbers of respiration/minute).

[c]Morning rectal temperature (^{o}C).

[d]Evening rectal temperature (^{o}C).

[e]Plasma protein:fibrinogen ratio.

[f]Total white blood cell counts (per mm^3).

[g]Absolute numbers of neutrophils (per mm^3).

[h]Absolute numbers of lymphocytes (per mm^3).

Table 8. Overall means for eight variables which were measured daily for infected and non-infected calves

	Infected	Non-infected
MR[a]	37.78	44.80
ER[b]	38.98	46.38
MT[c]	39.19	39.21
ET[d]	39.34	39.23
PP:F[e]	10.97	11.45
WBC[f]	12178	12775
AN[g]	7409	8306
AL[h]	4294	4097

[a] Morning respiration (numbers of respiration/minute).

[b] Evening respiration (numbers of respiration/minute).

[c] Morning rectal temperature (^{o}C).

[d] Evening rectal temperature (^{o}C).

[e] Plasma protein:fibrinogen ratio.

[f] Total white blood cell counts (per mm^3).

[g] Absolute numbers of neutrophils (per mm^3).

[h] Absolute numbers of lymphocytes (per mm^3).

reached around days 10 to 14 PI (5 to 9 days after stopping injections of dexamethasone).

Infection appeared to have a negative influence on the respiration rate as illustrated in table 8. The difference approached significant levels ($P<0.09$) when the overall means were analyzed.

Analysis of the overall means indicates that calf temperatures were increased by both dexamethasone and M. dispar treatments. The difference was significant for the dexamethasone effect ($P<0.01$) and approached significant levels for the infection effect ($P<0.08$). Morning and evening temperatures were similarly affected (Appendix table A2). A plot of evening temperature (Figure 5) illustrates the temperature changes during the experimental period. Temperatures were higher from day 6 (soon after stopping dexamethasone) to day 15. Peaks were observed on day 12.

The effect of infection on temperature was milder than the dexamethasone effect. It approached significant levels ($P<0.08$) for evening temperatures with a peak around day 12 ($P<0.05$). Additive effects, rather than interaction, were observed as a result of the combined effects of dexamethasone and infection on temperature.

The differences for both respiration and temperature were more pronounced in the evening measurements than in the morning measurements.

Hematology

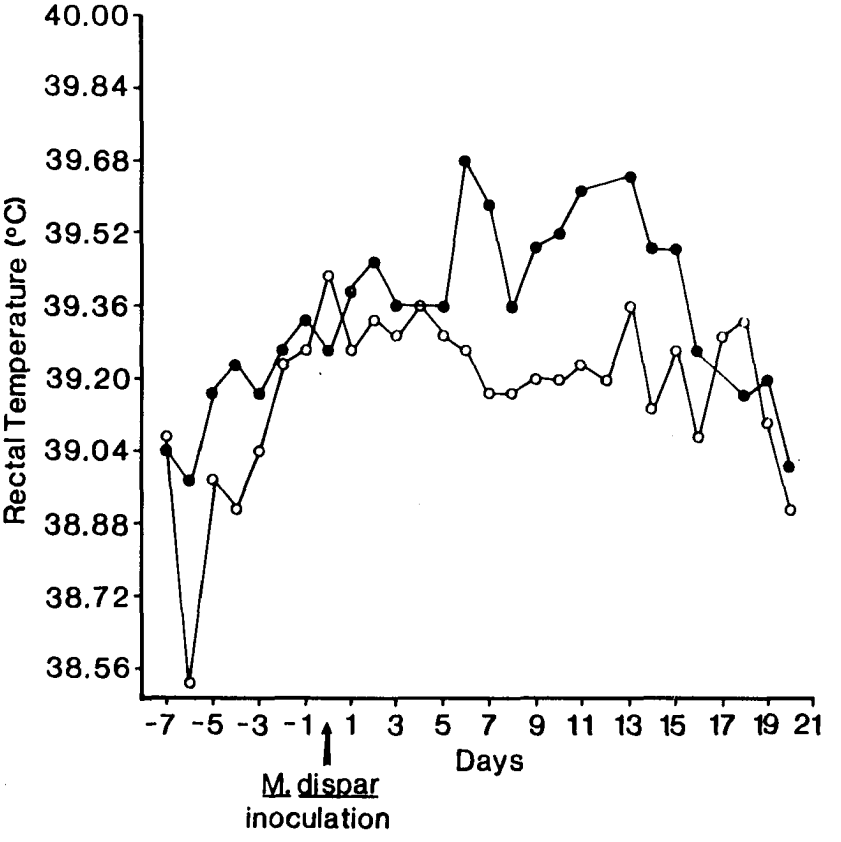
There was a highly significant difference in the total white blood cell counts and the differential leucocyte counts among dexamethasone treated and non-treated groups ($P<0.0001$). The differences included a

Figure 5. Effect of dexamethasone treatment on rectal temperature

Dexamethasome was administered from day -7 to day 5.

●—● Dexamethasome

o—o Saline


40.00
39.84
39.68
39.52
39.36
39.20
39.04
38.88
38.72
38.56
Rectal Temperature (°C)
-7 -5 -3 -1 1 3 5 7 9 11 13 15 17 19 21
Days
M. dispar
inoculation

decrease in the lymphocytic compartment ($P<0.001$) and a marked increase in the neutrophilic compartment ($P<0.0001$) resulting in an increase of total white blood cell counts. The increase of total white blood cell counts is indicated in figure 6, the increase of neutrophil counts is indicated in figure 7 and the decrease of lymphocyte counts is shown in figure 8.

The differences were detected 24 hours after the beginning of dexamethasone treatment and continued with just a slight daily decrease which was not enough to reach normal levels within the 10-day-period of dexamethasone injections (Figure 6). However, the total white blood cell and the neutrophilic compartments dropped to about normal levels 48 hours after stopping the dexamethasone injections (Figures 6 and 7). The peripheral lymphocyte numbers continued rather low for the duration of the experiment (Figure 8).

Dexamethasone also significantly decreased the plasma protein: fibrinogen ratio ($P<0.0002$). Figure 9 illustrates the PP:F ratio changes. These differences were due to highly increased total fibrinogen levels ($P<0.0001$) rather than differences in total plasma protein. The PP:F ratio was not significantly changed by the effects of infection. In fact, M. dispar infection did not have significant effects on any of the analyzed hematologic parameters.

Hemoglobin, packed cell volume, band neutrophils, monocytes and eosinophils did not present any apparent changes during the experiment and were not statistically analyzed.

Dexamethasone and infection effects, when present, were additive

Figure 6. Effect of dexamethasone treatment on the total white blood cell count

Dexamethasone was administered from day -7 to day 5

●—● Dexamethasone

o—o Saline

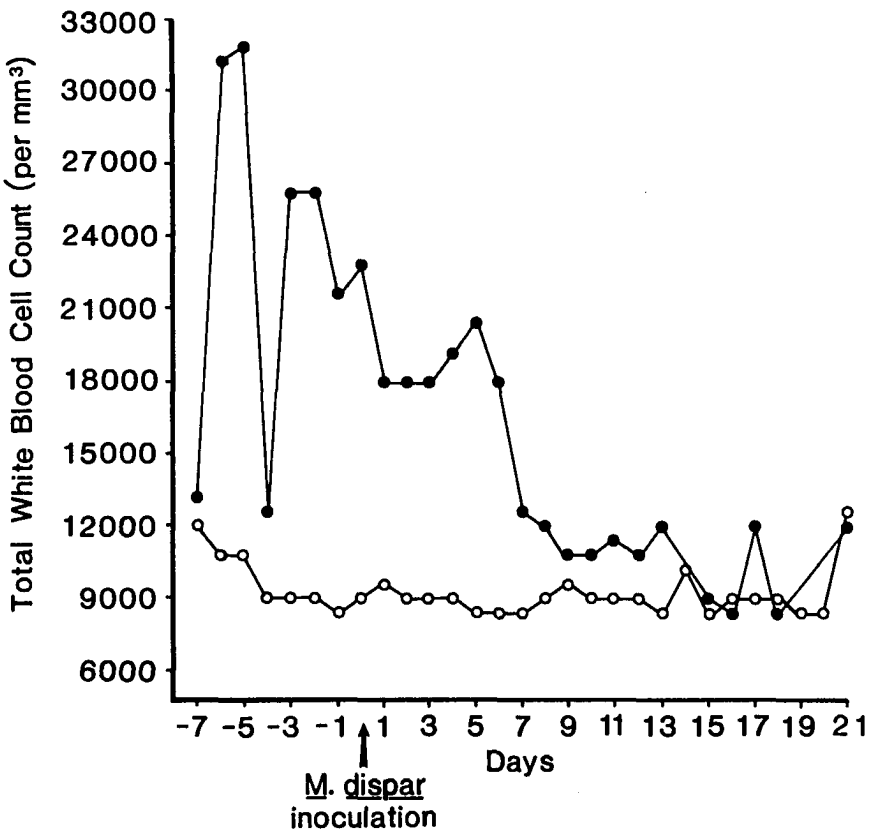

Total White Blood Cell Count (per mm³)
33000
30000
27000
24000
21000
18000
15000
12000
9000
6000
-7 -5 -3 -1 1 3 5 7 9 11 13 15 17 19 21
Days
M. dispar
inoculation

Figure 7. Effect of dexamethasone treatment on total neutrophil numbers per mm^3

Dexamethasone was administered on day -7 to day 5

●—● Dexamethasone

o—o Saline

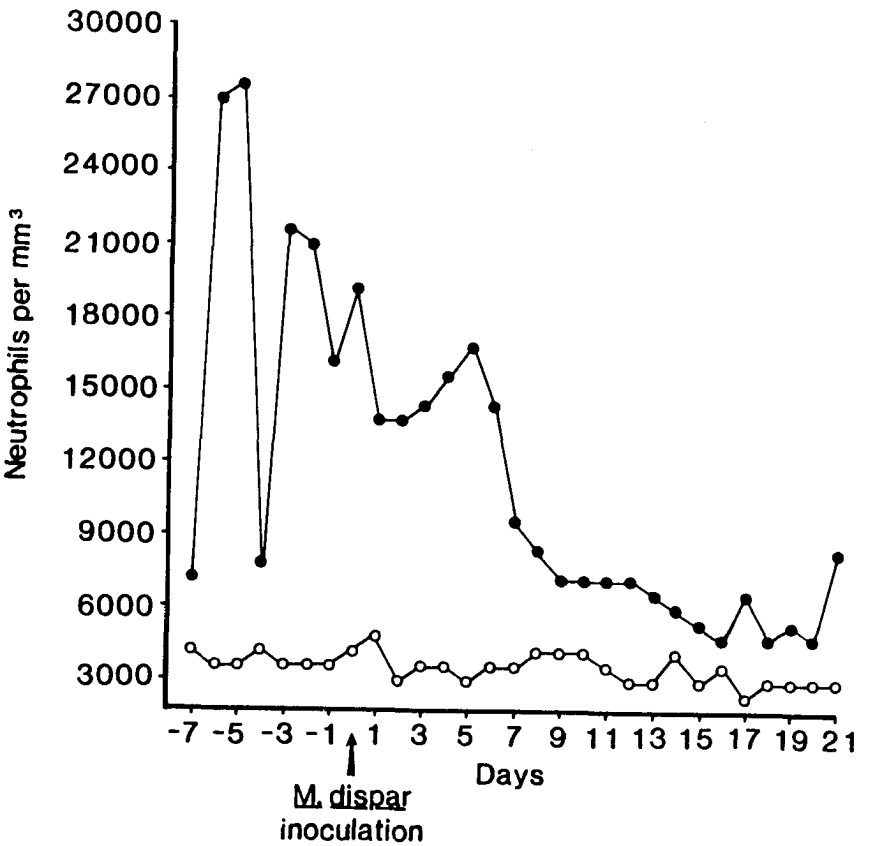

30000
27000
24000
21000
18000
15000
12000
9000
6000
3000
Neutrophils per mm³
-7 -5 -3 -1 1 3 5 7 9 11 13 15 17 19 21
Days
M. dispar
inoculation

Figure 8. Effect of dexamethasone treatment on the total lymphocyte numbers per mm^3

Dexamethasone was administered from day -7 to day 5

●——● Dexamethasone

o——o Saline

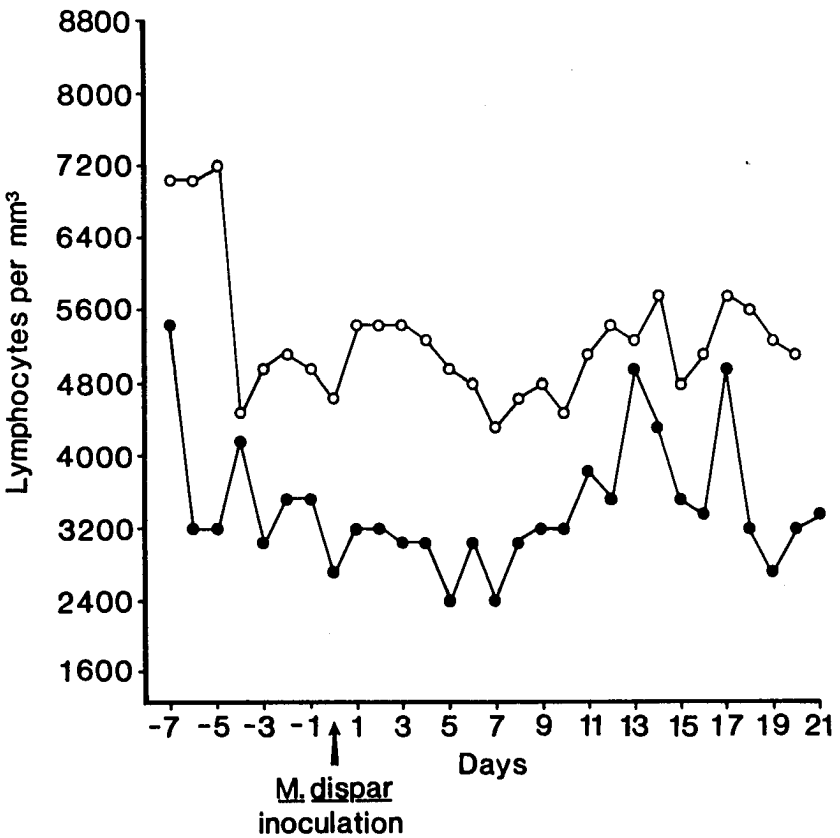
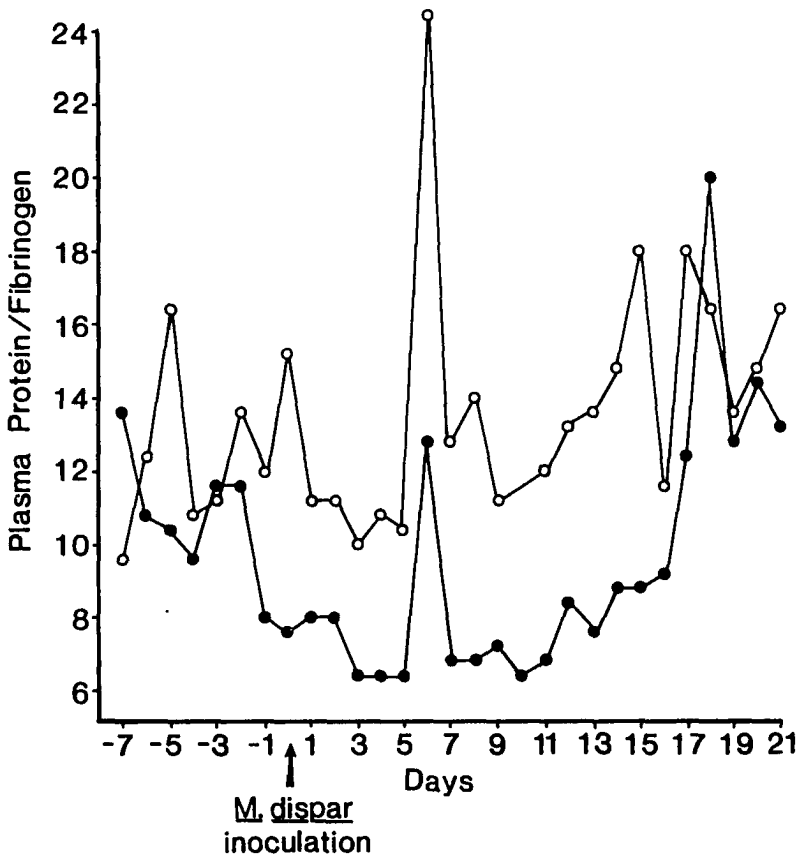

8800
8000
7200
6400
5600
4800
4000
3200
2400
1600
Lymphocytes per mm³
-7 -5 -3 -1 1 3 5 7 9 11 13 15 17 19 21
Days
M. dispar
inoculation

Figure 9. Effect of dexamethasone treatment on the plasma protein:fibrinogen ratio

Dexamethasone was given on day -7 to day 5

●——● Dexamethasone

o——o Saline


Plasma Protein/Fibrinogen
24
22
20
18
16
14
12
10
8
6
-7 -5 -3 -1 1 3 5 7 9 11 13 15 17 19 21
Days
M. dispar
inoculation

and no evidence of interaction could be found.

Pathology

Gross lesions

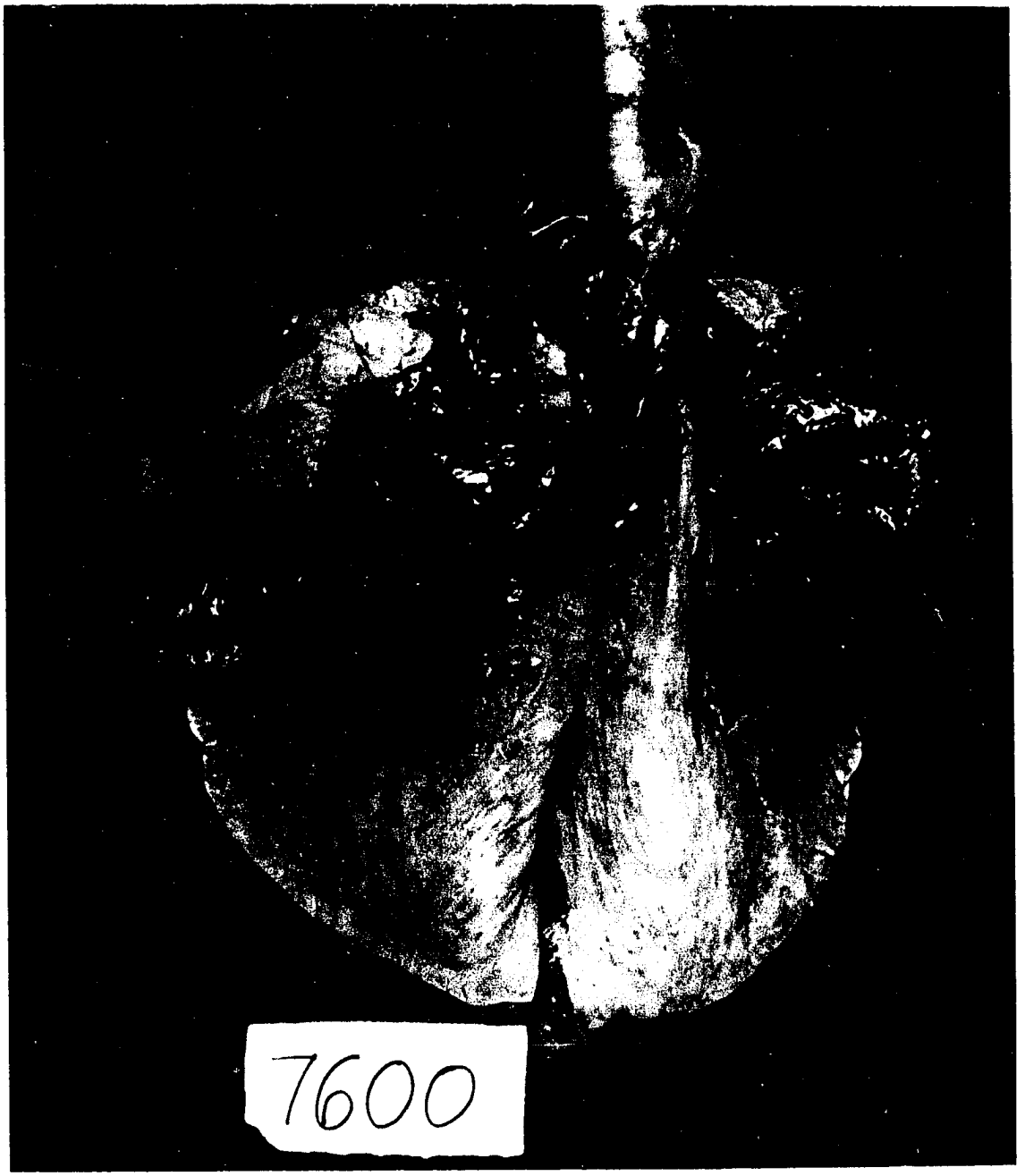
The most severe gross lesions were consolidation of the lung parenchyma in animals of experiment 1, all of which were infected with M. dispar. The lesions varied in extent and severity but were similar among all calves.

Calf 7600 (infected-dexamethasone) had severe changes in the right and left cranial lobes and in the right middle lobe. These lobes were diffusely involved and a small (1.5 X 3 cm) lesion was also present at the cranial edge of the left caudal lobe, adjacent to the cranial lobe (Figure 10). The involved areas of the lung were dark-red and consolidated. They had a firm consistency and no crepitation. There was a sharp demarcation between consolidated lobes or areas and adjacent normal tissue. Consolidated tissue sank promptly when placed into a bottle containing formalin solution. The cut surfaces had marked edematous thickening of interlobular septa. A small amount of whitish exudate could be expressed from the bronchi. The bronchial and mediastinal lymph nodes were enlarged, edematous and dark-red.

Calves 7102 and 7109 (infected-dexamethasone) had extensive lesions. Calf 7102 had more than one half of the cranial part (edge portion) of the right cranial lobe affected.

Figure 10. Gross lung lesions of a Mycoplasma dispar infected calf (7600) which was treated with dexamethasone

Calf was killed sixteen days post inoculation (eleven days after stopping the 10-day-period of dexamethasone injections)


7600

Calf 7109 also had the right cranial lobe similarly affected. In addition, the anteroventral one-half of the caudal part of the left cranial lobe (edge portion as well) was diffusely reddened and the remaining lung parenchyma had multiple minute gray foci on the pleural surface. Even though they were much smaller and less severe, the areas of consolidation in the lungs of these two calves had the same characteristics as the lesions described for calf 7600.

Calves 7104 and 7599 (infected-saline) had focal areas of parenchymal consolidation. Calf 7104 had two small lesions of about 2 cm in diameter with one in the caudal part of the left cranial lobe and the other in the cranial part of the left caudal lobe. Calf 7599 had similar lesions in the right middle lobe and adjacent to the cranial edge of the left caudal lobe. These were actually clusters of small (1 to 2 cm) lesions with three lesions in the first site and three in the second.

Different patterns of gross lesions were occasionally observed in other animals. Calf 72 (infected-dexamethasone) had multiple dark foci of a few mm to about 3 cm involving the cranial lobes, the right middle lobe and large areas of the anteroventral aspects of the caudal lobes. Most of these lesions were sharply demarcated from adjacent normal tissue. The main bronchi were plugged with a firm, granular, caseous material which appeared to have come down from a fistulous abscess in the upper trachea. The trachea itself contained the granular material on the mucosal surface which otherwise appeared normal.

Calf 7103 (infected-dexamethasone) had several small, dark, grayish consolidated areas of nodular appearance on the lung surface. These lesions, though small, were associated with a more diffuse redness of a considerable portion of the adjacent parenchyma. The tissue was hypocrepitant and had a dried appearance when cut.

Calf 7105 (infected-saline) also had a few consolidated areas involving the cranial lobes and a diffuse redness of the parenchyma which involved most of the lung, including large portions of the anteroventral aspects of the caudal lobes.

Calf 98 (infected-saline) had a 1.5 cm lesion in the cranial part of the right cranial lobe. The lesion was pinkish-red and well demarcated from the surrounding tissue.

None of the remaining calves had grossly observable lung lesions.

Calf 101 (infected-saline) and calf 102 (infected-dexamethasone), both of experiment 3, had grossly observable lesions in the kidneys. Lesions were more prominent in calf 101 and consisted of multiple greyish-white nodules which were of 0.5 to 1 cm in diameter and visible through the kidney capsule. The nodules were confined to the cortex and some projected slightly from the surface.

Histopathology

Multiple, minute, granulomatous lesions were observed in 9 dexamethasone treated calves.

Calf 7109 had more than 200 granulomas in 26 lung sections examined and calf 7103 had over 100 granulomas in 33 lung sections.

The other animals (calves 24, 31, 57, 72, 73, 75 and 7600) had a few to many granulomas each. These lesions in all animals were remarkably similar to those seen in calves 7103 and 7109. Figures 11 and 12 illustrate the pattern of these lesions.

The large number of granulomas observed in calves 7103 and 7109 allowed an evaluation of different stages of granuloma formation. Many of the granulomas seemed to be initiated in the lumen of small bronchi and bronchioles because the center of the more immature ones had a recognizable bronchus or bronchiole which had undergone caseation necrosis. The fibrous connective tissue surrounding the granulomas appeared in some cases to be a proliferation of pre-existing peribronchiolar connective tissue. In the older and more organized granulomas, the center was composed of a small cluster of neutrophils surrounding, in many instances, fragments of fungal hyphae (Figures 11 and 12). These small central clusters were then wrapped by a broader sheath of lymphocytes, histiocytes, fibrous connective tissue, occasional giant cells, and scattered neutrophils. Granulomatous lesions which originated in small groups of alveoli could also frequently be seen (figure 12). Several small granulomas further coalesced to become larger granulomatous masses. Sections stained by the Gomori's methenamine silver method usually revealed fragments of fungal hyphae (Figure 11B). In addition, different species of Aspergillus were isolated from the lungs of some of the experimental calves which included some of those having granulomatous lesions (Table 4).

One small granuloma each was observed in the lungs of two saline-

Figure 11. Granulomas in lungs of dexamethasone treated calves

A. Well-formed granuloma

Notice a central zone of neutrophils (arrow) - Calf 7103

Hematoxylin and eosin stain

B. Granuloma with numerous fungal hyphae. Inset: higher magnification

Gomori's methenamine silver stain

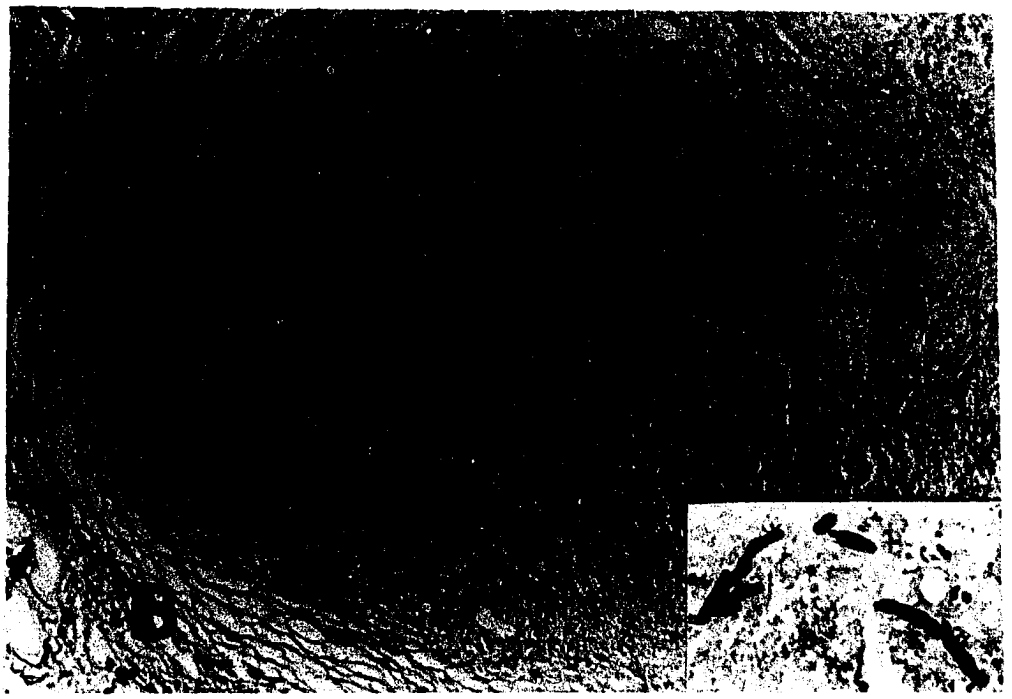
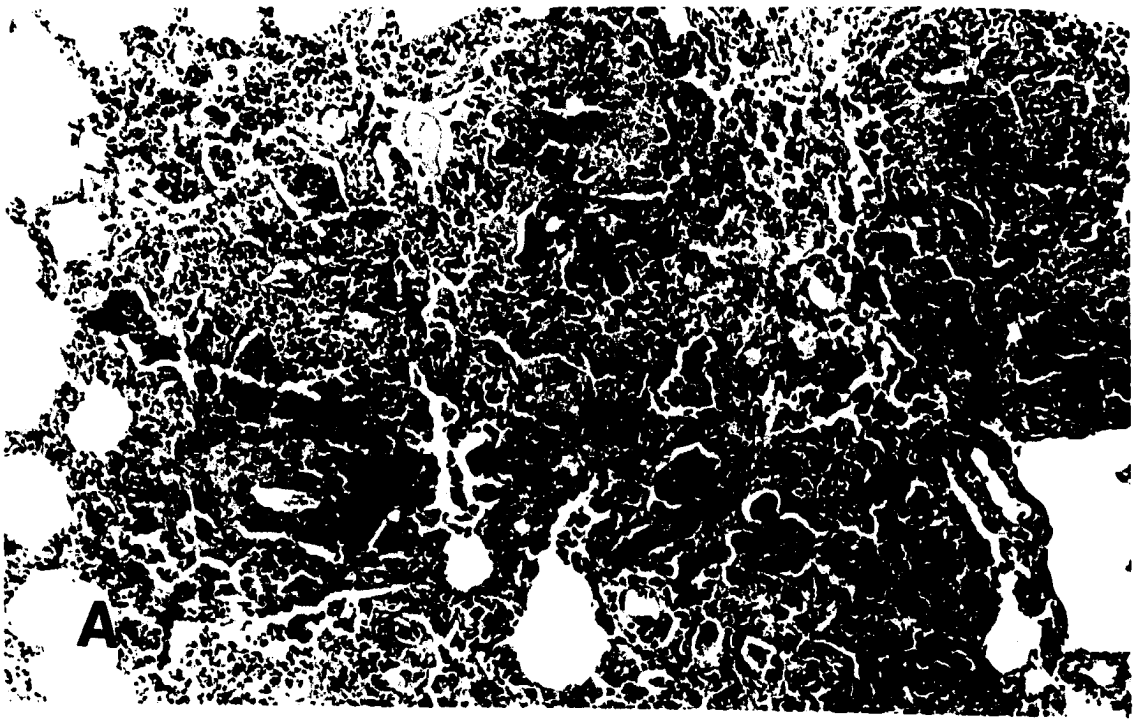


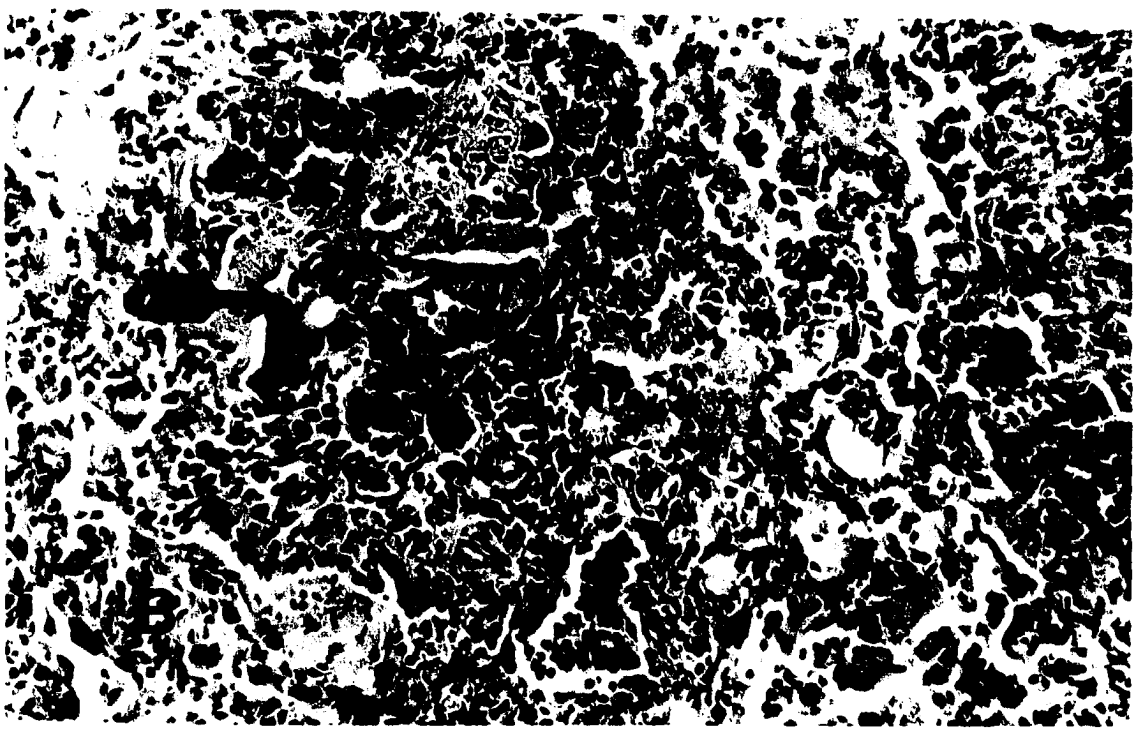
Figure 12. Granulomas in lungs of a dexamethasone treated calf (7103)

A. Early granuloma formation

B. Higher magnification of A. Notice a minute rosette which is a cross section of fungal hyphae surrounded by club shaped structures and early tissue reaction

Hematoxylin and eosin stain


A


B

treated animals (calves 37 and 7105). These granulomas were rather immature in contrast to the majority of those observed in dexamethasone treated animals.

A range of histopathologic lesions which may have been associated with the mycoplasma infection was evaluated and utilized for scoring the lesions of the respiratory tract. Table 9 indicates that a single score was given for lesions in each of turbinate, pharynx and trachea. Four different types of lung lesions were scored individually and an overall score was also assigned to represent the extent of the lung lesions.

Analysis of variance of overall lung lesion scores indicated that dexamethasone had no significant effect ($P<0.19$) whereas infection with M. dispar approached significance ($P<0.06$).

Pulmonary lesions will be described for individual calves or groups of calves in the next few paragraphs.

Calf 7600 (infected-dexamethasone) had the most extensive and striking lesions. The sharp demarcation seen grossly was also apparent histologically. Sections coming from the cranial and middle lobes were markedly affected but those from caudal lobes, even from areas immediately adjacent to an affected area, had only mild changes.

The lesions observed in consolidated lobes were characterized by a heavy neutrophilic infiltrate both in the airways and in the alveolar spaces. Neutrophils were almost the sole component of the bronchial exudate but they were mixed with fibrin and macrophages in alveolar spaces. The bronchi and bronchioles had variable degrees of epithelial

Table 9. Lesion scores for histopathological lesions of the respiratory tract for infected and non-infected calves under dexamethasone and saline treatments

Infective status	Calf No.	Treatment	Day PI[a] animal was necropsied	Turbinate score	Pharynx score	Trachea score
	7103	DEX[f]	11	0	ND[g]	1
	57	DEX	12	1	1	0
	7102	DEX	12	2	ND	3
	72	DEX	16	1	ND	2
	7109	DEX	16	4	ND	2
	7600	DEX	16	4	ND	4
	102	DEX	20	1	ND	0
	75	DEX	21	2	0	2
infected	$\bar{x}$			1.9		1.7
	37	SAL[h]	12	0	0	0
	7105	SAL	12	0	ND	0
	7599	SAL	12	2	ND	3
	74	SAL	16	2	1	1
	7104	SAL	16	3	ND	3
	7108	SAL	16	1	ND	3
	98	SAL	20	1	2	0
	101	SAL	20	1	1	0
	76	SAL	21	1	1	0
	$\bar{x}$			1.2		1.1
	24	DEX	11	0	ND	0
	14	DEX	20	1	ND	1
	31	DEX	20	0	ND	0
non-	73	DEX	21	1	0	0
infected	$\bar{x}$			0.5		0.3
	17	SAL	12	0	0	0
	12	SAL	20	0	0	0
	77	SAL	21	0	0	0
	$\bar{x}$			0	0	0

[a]Post inoculation.
[b]Bronchial associated lymphoid tissue.
[c]Interstitial thickening.
[d]Alveolar infiltrate.
[e]Overall lesion scores.
[f]Dexamethasone.
[g]Not done.
[h]Saline.

Lung scores						
BALT[b] hyperplasia	Bronchitis & Bronchiolitis	IT[c]	AI[d]	OLS[e]	Number HE sections examined	Number of granulomas
0	3	3	1	1	33	107
0	1	2	1	1	51	8
2	2	2	2	1	32	0
2	2	3	3	2	37	4
1	2	3	2	1	26	217
3	4	4	4	3	42	14
0	0	2	1	1	40	0
0	1	2	2	1	42	3
1.0	1.9	2.6	2.0	1.4	37.9	44.1
0	0	3	2	1	44	1
0	0	4	2	1	27	1
1	1	2	0	0	26	0
4	2	3	2	1	40	0
3	2	4	0	1	28	0
0	0	2	0	0	22	0
2	2	4	0	1	39	0
1	0	3	0	1	39	0
4	0	2	1	1	45	0
1.7	0.8	3.0	0.8	0.8	34.4	0.2
0	0	1	1	0	39	3
0	0	3	1	1	35	0
0	1	2	1	1	39	3
0	0	1	1	0	45	1
0	0.3	1.7	1.0	0.5	33.5	2.0
2	1	2	1	1	27	0
0	0	1	0	0	38	0
2	0	2	1	1	44	0
1.3	0.3	1.7	0.7	0.7	36.3	0

hyperplasia. Their mucosae were infiltrated by numerous neutrophils. Neutrophils and mononuclear inflammatory cells were also in high numbers in the submucosa and lamina propria of the bronchi and bronchioles as well as in the interstitial tissue. The degree of lymphoid infiltration around bronchi and bronchioles and in the lamina propria was of a mild nature, did not form follicles, and usually was in association with the lesions just described. This warranted a classification of severe bronchitis and bronchiolitis with accompanying alveolitis.

Fibrin was the major component of the alveolar exudate but most of it was in a process of being digested by macrophages which were also present in large numbers. The exudative process was in a resolving stage.

Edema was noticed around some blood vessels and was particularly marked in the interlobular septa which were greatly thickened and had many thrombosed lymphatic vessels. The entire lesion complex was one of consolidated lobules and subacute inflammation with fibrinopurulent bronchopneumonia.

There was diffuse interstitial thickening of mild degree throughout the caudal lobes. Neutrophil infiltration of the interstitial tissue was mild and it was almost completely absent from the airways and alveolar spaces.

Fourteen granulomas were counted in the 42 lung sections examined.

Bronchopneumonia in two more animals (calf 7103, infected-dexamethasone; and calf 7109, infected-saline) was very similar histologically

but was less extensive, involving only consolidated lobes or consolidated portions of a lobe, and was milder in degree.

Thirteen animals (calves 12, 14, 31, 72, 74, 75, 98, 102, 7102, 7104, 7105, 7108 and 7599) had an even milder bronchiolitis. Only three of these animals (calves 12, 14 and 31) were non-infected. The lesions in these 13 calves were limited to particular lobes or areas and involved a few airways. The extent of the lesions varied from animal to animal but was generally characterized by some degree of bronchial epithelial hyperplasia, bronchiectasia and scattered neutrophils which were among the mucosal infoldings, among the epithelial cells and in the submucosa. The alveolar spaces of these areas frequently had slightly increased numbers of alveolar macrophages and an occasional neutrophil. Figure 13 illustrates the alveolar, bronchiolar and bronchial lesions as observed in calf 7102 which had the most pronounced lesions among these 13 calves.

Interstitial thickening was present in most of the experimental animals to a variable extent. Only two animals (calf 24, non-infected-dexamethasone; and calf 12, non-infected-saline) were considered to have alveolar walls of normal thickness.

The alveolar spaces of all calves contained variable numbers of free macrophages. The macrophages were often seen in small groups of alveoli throughout the lung sections or in a more diffuse pattern. Occasionally they were associated with a small amount of fibrin and a few neutrophils. Those calves with more pronounced airway lesions also had increased numbers of macrophages in the associated alveoli.

Figure 13. Histological sections of lungs of a *Mycoplasma dispar*-infected calf (7102) which was treated with dexamethasone

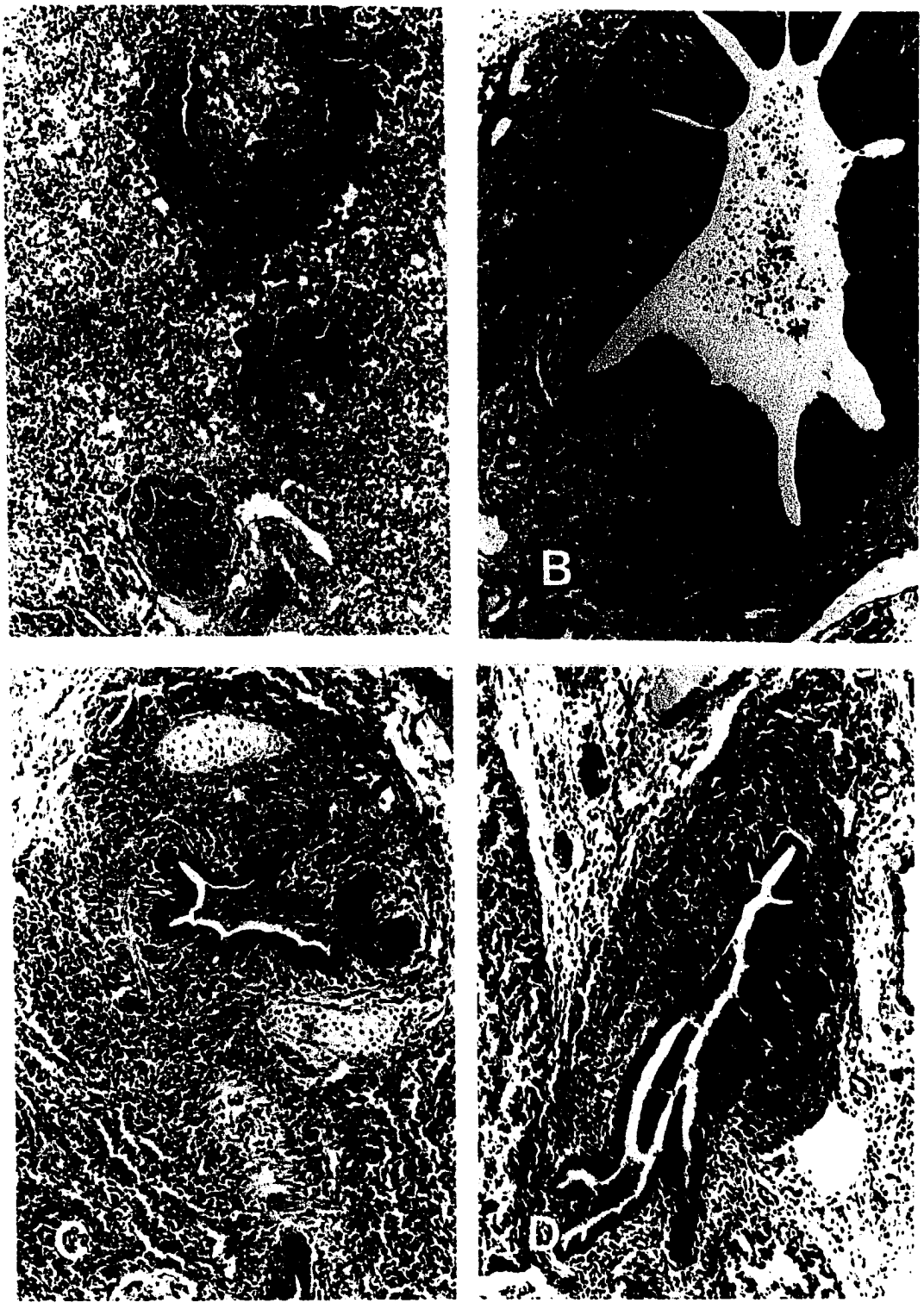
A. Consolidated lobule with bronchioles and alveoli containing inflammatory exudate

B. Bronchial wall diffusely infiltrated by mononuclear inflammatory cells

C. Bronchitis. Mononuclear inflammatory cells predominate in the bronchial wall and neutrophils in the lumen

D. Bronchiolitis. Mononuclear cells form a diffuse, loosely arranged cuff around the bronchiole

Hematoxylin and eosin stain


B
C
D

Some of the best examples of peribronchial and peribronchiolar lymphoid hyperplasia were observed in calf 17 (non-infected-saline), calf 76 (infected-saline) and calf 77 (non-infected-saline) which had very mild parenchymal lesions. These 3 calves had an active proliferation of lymphoid tissue to the extent of follicular formation and some lymphoid invasion of the submucosa and lamina propria throughout several smaller and larger airways.

It should be noted that mycoplasma was demonstrated in the upper respiratory tract (trachea and pharynx) of calf 76 by both FAT and electron microscopy but there was no evidence of the organism in his lungs. The other two calves (17 and 77) had negative results for all tests performed. It is true, however, that hyperplasia of the peribronchial and peribronchiolar lymphoid tissue was considerable in several infected animals. Its association with rupture of the basement membrane, active proliferation into the lamina propria and occasional perivascular accumulations of lymphocytes contributed to the high scores applied to this segment of the histological evaluation of the lungs of calves 74, 98, 7102, 7104 and 7600 (Table 9).

Dexamethasone treated calves, as illustrated in table 9 ("BALT hyperplasia" column), had less bronchial associated lymphoid tissue than saline-treated animals, even though calves with the most extensive lung lesions were among the dexamethasone-treated ones. Interstitial thickening appeared to bear a similar relationship. Figures 14, 15 and 16 illustrate the peribronchial and peribronchiolar lymphoid arrangement in lungs of infected calves under both saline and dexamethasone

Figure 14. Histological sections of lungs of calves treated with saline and infected with *Mycoplasma dispar*

A. Calf 7105. Peribronchial lymphoid hyperplasia and interstitial thickening of parenchyna adjacent to the bronchus

B. Calf 7599. Hyperplasia and invasion of the peribronchial lymphoid tissue into the lamina propria

C & D. Calf 76. Peribronchiolar lymphoid hyperplasia Notice peribronchiolar lymphoid tissue displaying follicular arrangement and invasion of the lamina propria

Hematoxylin and eosin stain

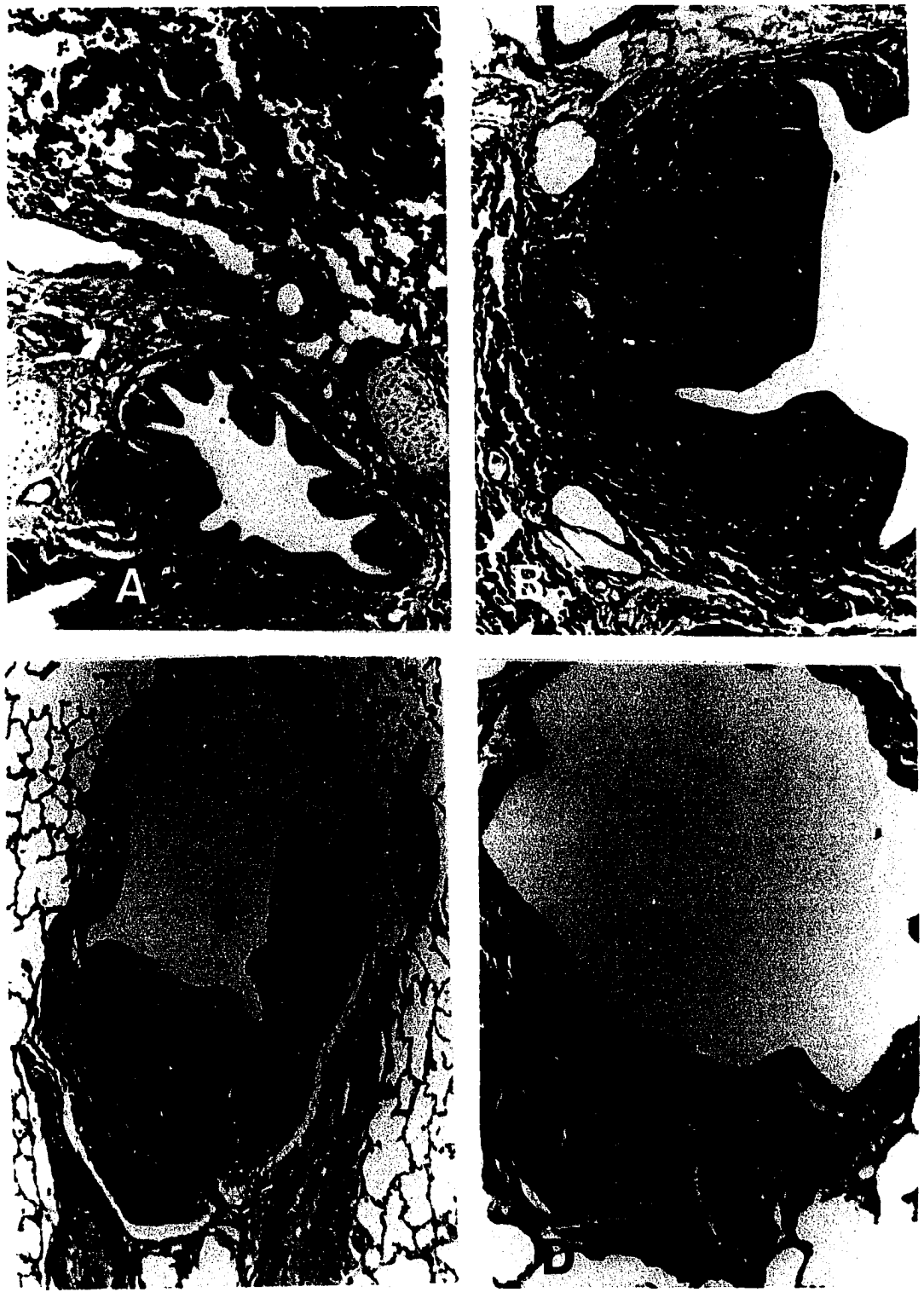

A
B

Figure 15. Histological sections of lungs of animals treated with saline and infected with *Mycoplasma dispar*

A. Calf 74. Peribronchiolar and perivascular lymphoid accumulation

B. Calf 7599. Lymphocyte and neutrophil infiltration of the lamina propria of a large bronchus. Note neutrophils among epithelial cells (arrow)

C & D. Calf 76. Peribronchiolar and perivascular infiltrations of lymphocytes

Hematoxylin and eosin stain

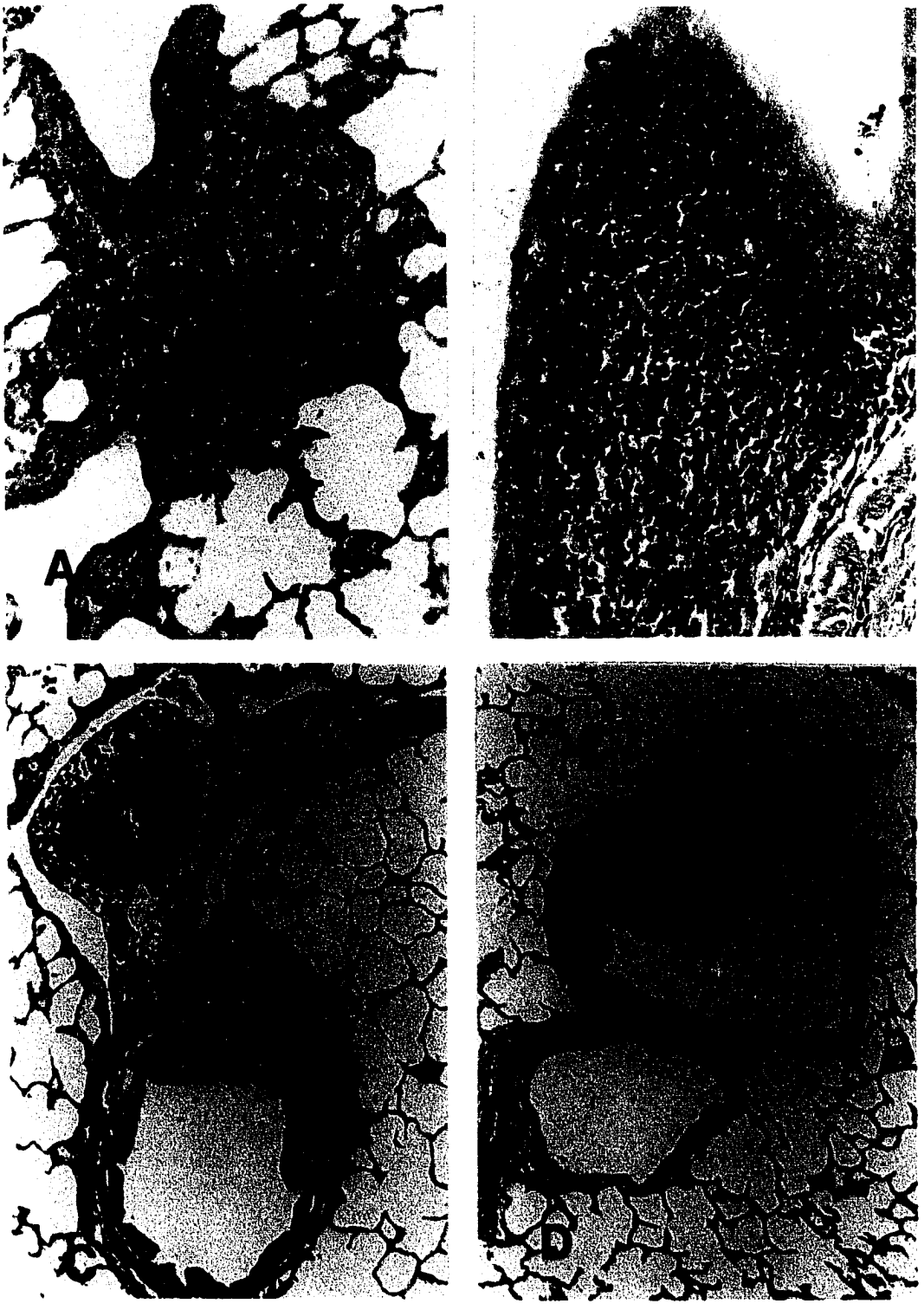

A
B

Figure 16. Histological sections of lungs of calves which received different treatments

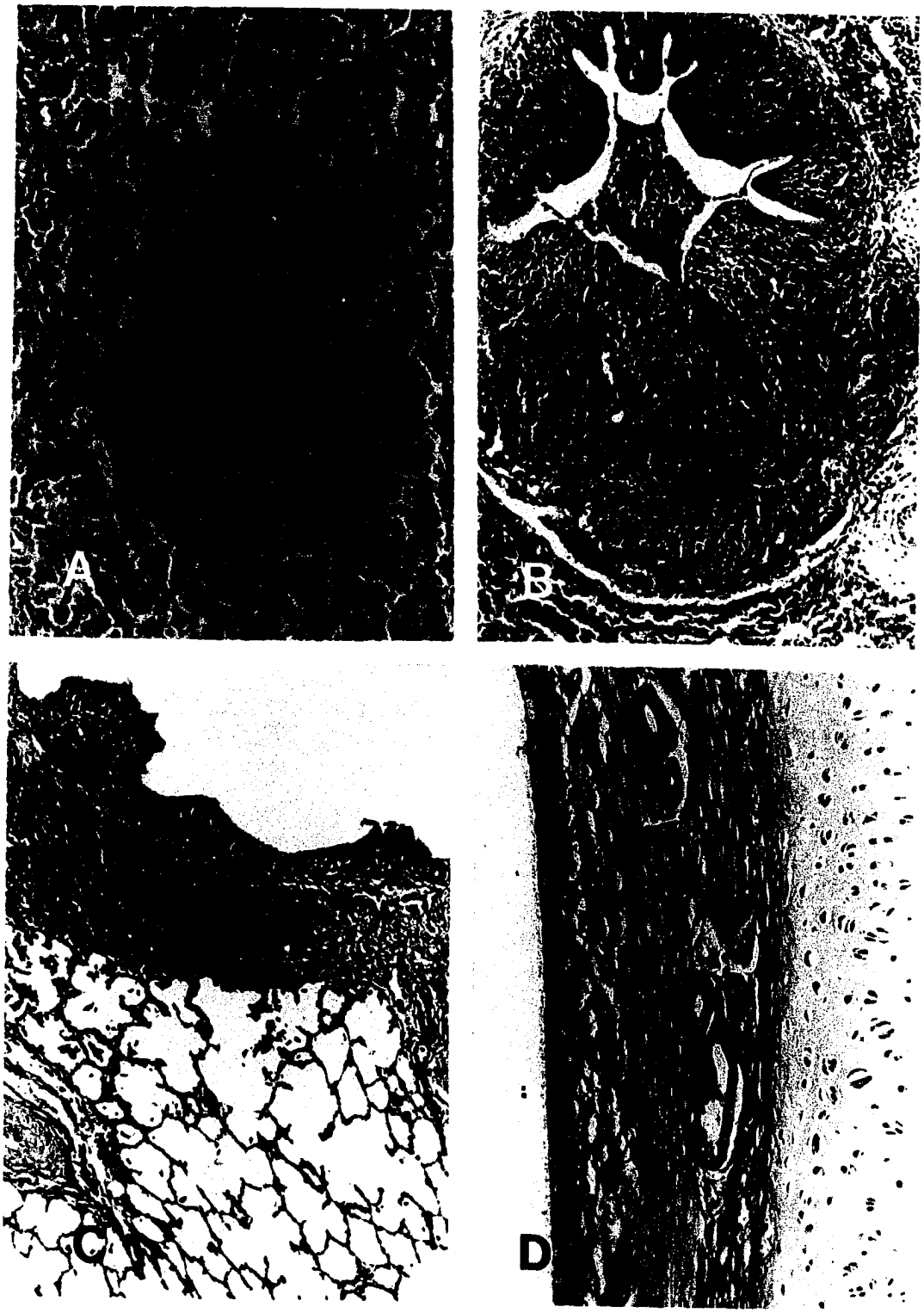
A. Calf 7600 (infected-dexamethasone). Bronchiolitis and alveolitis. Luminal infiltrate is composed of neutrophils. Parenchymal reaction includes both lymphocytes and neutrophils

B. Calf 7599 (infected-saline). Lumen of the bronchus contains a neutrophilic infiltrate. The peribronchial lymphoid tissue is hyperplastic and invades the lamina propria

C. Calf 77 (non-infected-saline). Normal peribronchial lymphoid tissue surrounging a normal bronchus

D. Higher magnification of another segment of the bronchial mucosa of C (Same bronchus with infiltration)

Hematoxylin and eosin stain


A
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treatments. Other aspects of lesions already described are also indicated in those figures.

The results of tracheal lesion evaluations and microbiological isolations are shown in table 9.

Tracheas yielding the highest mycoplasma titers were generally those which displayed the most pronounced lesions, except for calf 7105 (infected-saline) which had a high M. dispar titer with no lesion and calf 7104 (infected-saline) which had lesions without an apparent high M. dispar titer in the trachea. The M. dispar titer of other sites of the respiratory tract of calf 7104 was high, however.

The tracheal lesions were frequently a mild to pronounced infiltration of the lamina propria by mononuclear inflammatory cells and neutrophils (Figure 17). The mucosa had a variable degree of neutrophil infiltration among the epithelial cells, and the neutrophils occasionally formed small clusters. There were also recognizable changes in the cilia of the mucosal cells. The changes consisted of clumping of cilia which were frequently observed in patchy areas. The epithelium was often hyperplastic and had multiple layers. Loss of cilia was sometimes seen in these areas and usually was associated with degenerative changes of the outer-most layer of epithelial cells. No erosions, plaques, or free exudate were noticed.

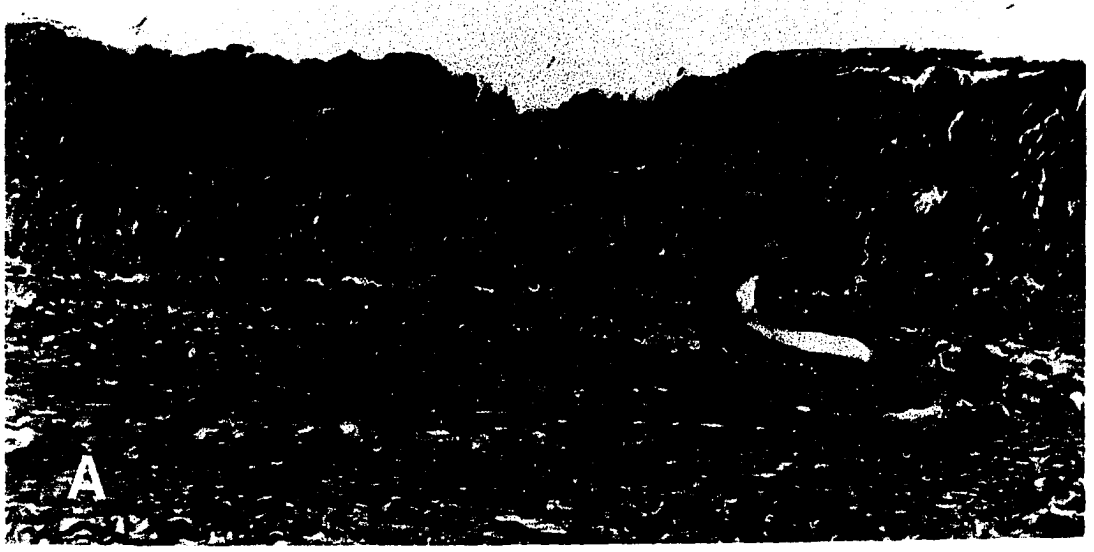
The turbinate lesions as evaluated histologically (table 9) paralleled the tracheal lesions. The same calves which had a high frequency of mycoplasmas in the trachea, pharynx or nasal swabs were the ones with more pronounced turbinate lesions. The lesions were

Figure 17. Histological sections of tracheas of calves which received different treatments

A. Calf 75 (infected-dexamethasome)
Extensive loss of cilia, mononuclear inflammatory cells, and neutrophil infiltration of lamina propria. Note hyperplastic epithelium indicated by numerous layers and superficial erosion

B & C. Calf 7104 (infected-saline)
Neutrophil and lymphocyte infiltrations of the lamina propria. There is also clumping and loss of cilia

Hematoxylin and eosin stain


A


B


C

basically variable degrees of infiltration of mononuclear inflammatory cells and neutrophils in the lamina propria (Figure 18). There was also mucosal cell vacuolation with neutrophils scattered among epithelial cells.

Three infected-dexamethasone calves (7102, 7109 and 7600) and one infected-saline calf (7104) which were all from experiment 1 had occasional small clumps of neutrophils in vacuoles of the nasal mucosa (microabscesses) (Figure 18A).

Lesions seen in several cases also included clumping of cilia, occasional loss of cilia and sometimes degenerative changes which progressed to minute focal areas of necrosis of the epithelium.

The eleven calves from which complete sets of tissues were available for evaluation of the pharynx are shown in table 9.

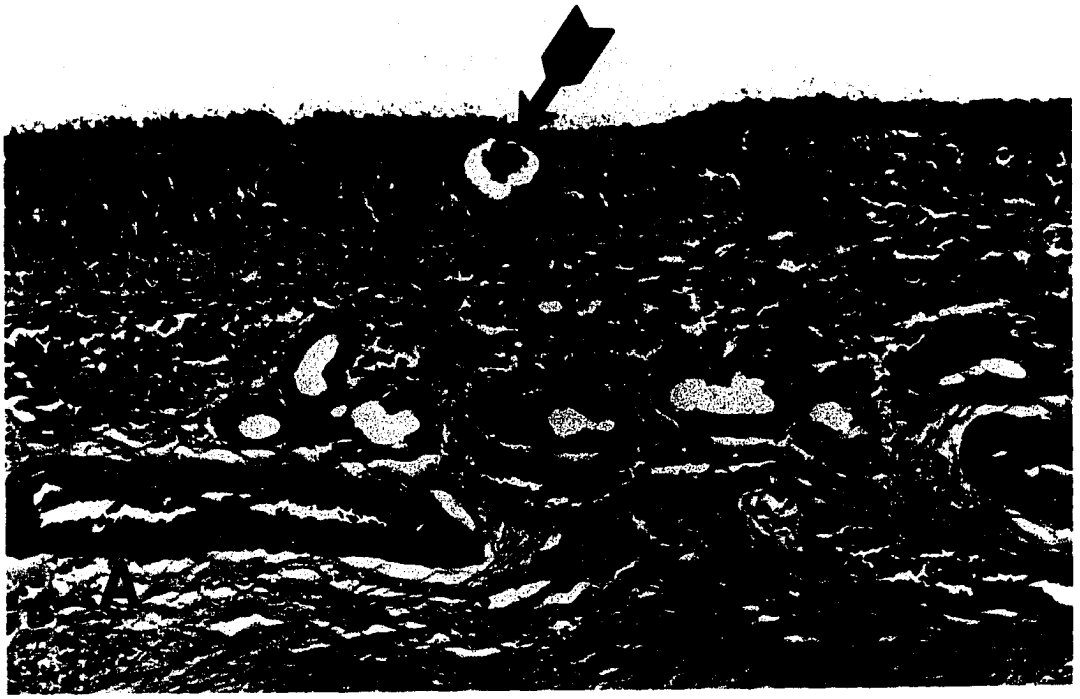
Calf 98 (infected-saline) had the most pronounced changes in the tonsilar crypts which had frank neutrophilic exudate and cellular debris. A few focal areas of epithelial necrosis and pronounced vacuolation of crypt epithelium were present. There were scattered neutrophils among epithelial cells of the crypts. The pharyngeal mucosa also had focal areas with degenerative changes of the outermost epithelial layers. A few neutrophils were scattered among epithelial cells. The nasopharyngeal area had slight mononuclear inflammatory cell and neutrophil infiltration in the lamina propria, slightly increased numbers of goblet cells and visible clumping and loss of cilia throughout the mucosa. These changes warranted a score of 2 on the overall evaluation of the pharyngeal changes (Tables 4 and 9).

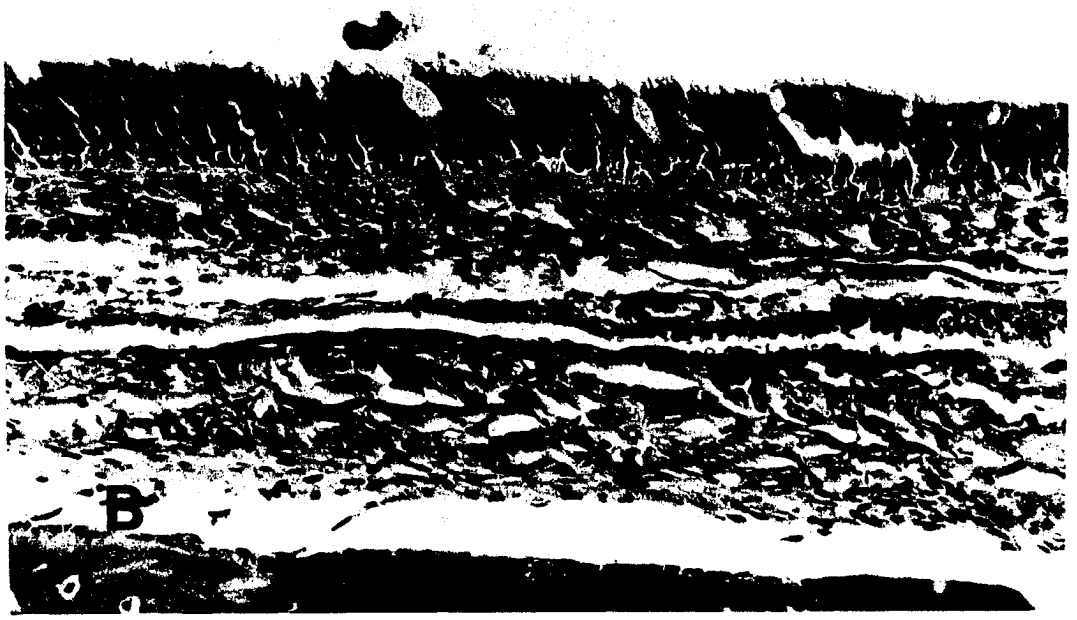
Figure 18. Histological sections of nasal turbinates of Mycoplasma dispar infected calves which were on dexamethasone treatment

A. Calf 7600. Clumping and loss of cilia Lymphocyte and neutrophil infiltrations of the mucosa and lamina propria. A micro-abscess can be seen in the mucosa (arrow)

B. Calf 75. Lymphocyte and neutrophil infiltrations of the mucosa and lamina propria Notice increased numbers of goblet cells

Hematoxylin and eosin stain




B

Calves 57 (infected-dexamethasone), 74, 76 and 101 (infected-saline) which were all infected with M. dispar as demonstrated by FAT, microbiological isolations and electron microscopic demonstration of mycoplasma, had about the same kinds of pharyngeal changes but to a milder degree designated by a score of 1.

Calf 75 (infected-dexamethasone) and calf 37 (infected-saline) which were infected with M. dispar; calf 73 (non-infected-dexamethasone), and calves 12, 17 and 77 (non-infected-saline) had no oropharyngeal or nasopharyngeal changes. The histological score for the pharynx of each of these 6 calves was zero.

Following are descriptions for specific sites of pharyngeal tissues.

The non-keratinized stratified squamous epithelium of the oropharynx was evaluated in 21 calves. No significant lesion was found in that tissue, except for the tonsilar crypts of calf 98 as described previously.

The epithelium of the tonsilar crypts and sinuses of eleven infected and six non-infected calves were examined. The tonsilar crypts all had some degree of neutrophilic infiltration and debris accumulation. However, these changes were most pronounced in five infected calves which were treated with saline. These calves were 74 and 76 of experiment 4, calf 98 of experiment 2, calf 101 of experiment 3 and calf 7104 of experiment 1. Similar changes were also noticed in one non-infected animal (calf 14, non-infected-dexamethasone).

The nasopharynx was evaluated in thirteen animals. Slight changes were observed in most of them. Only a few of the infected

animals (calf 57, infected-dexamethasone; calves 74, 76, 98 and 101, all infected-saline) had more pronounced lesions which consisted of mononuclear inflammatory cells and neutrophilic infiltration of the lamina propria, epithelial vacuolation with scattered neutrophils in the epithelium, and some clumping and loss of cilia.

Variable degrees of lymphoid depletion were observed in the thymus, lymph nodes, spleen and tonsils of most of the dexamethasone-treated calves. Some of the animals had an atrophic thymus which was composed almost exclusively of stroma and was completely devoid of lymphocytes (Figure 19A). The lymph nodes in the same animals had poorly defined germinal centers (Figure 19B). The spleen also had marked lymphoid depletion and a relative increase in fibrous connective tissue. The tonsils had the same decrease in lymphocyte population. These lesions were particularly striking in calves 7102, 7109 and 7600 (Figure 19A and 19B) which were treated with dexamethasone and were infected with M. dispar.

Overall evaluation of the bronchial associated lymphoid tissue (BALT) was also interesting (Table 9). Dexamethasone appeared to have a considerable effect on BALT. The amount and arrangement of BALT was more prominent among infected animals which were treated with saline. The infected calves which were treated with dexamethasone had a reasonable amount of lymphoid tissue around bronchi and bronchioles but rarely were organized follicles present.

The two calves which had gross kidney lesions (101 and 102) and a large proportion of the experimental calves with grossly normal kidneys

Figure 19. Lymphoid depletion of thymus and lymph node of a dexamethasone treated (Mycoplasma dispar-infected) calf (7600)

A. Thymus. Severe lymphoid depletion Atrophy and relative increase of fibrous connective tissue

B. Lymph node. Lymphoid depletion with a lack of follicular arrangement

Hematoxylin and eosin stain


A


B

had microscopic lesions. The most frequent changes were focal interstitial fibrosis and slight mononuclear inflammatory cell infiltrates which varied from minute foci to extensive areas involving most of the cortex. The minute lesions were a zone of a few layers of mononuclear cells surrounding a tubule or a small group of tubules and an occasional glomerulus.

The larger areas as seen in calves 101 and 102 which had gross lesions were characterized by an edematous fibrous component and few mononuclear inflammatory cells. Tubules and glomeruli in those areas were shrunken and compressed by fibrous connective tissue.

Another type of lesion was a thickening of the Bowman's capsule of a few glomeruli which had an occasional progression to a fibrous scar without any inflammatory reaction.

Several sections of kidney were stained by Brown and Brenn and Warthin-Starry method and examined for the presence of common bacteria and spirochetes. None of these organisms could be observed.

DISCUSSION

Reproduction of mycoplasma pneumonia in calves has not been easily accomplished and the results of several investigations have been inconsistent. Most researchers have reported the reproduction of a mild pneumonia which lacked clinical signs. The present investigation dealt with a similar situation where severity of the disease was inconsistent among experiments. Inconsistent nature of the disease also prevented a definite evaluation of differences regarding lesion development. Differences among the individual experiments apparently influenced lesion severity more than postinoculation time, inoculation procedure or amount of inoculum. Some calves killed at 16 days PI had more pronounced lesions than calves killed at 11, 16, 20 or 21 days. This raised the question of whether other undetectable variables operated in those experiments in which lesions were more pronounced.

Experiments 1 and 3 were characterized by profuse shedding of M. dispar while calves from experiments 2 and 4 never shed any organism (with a single, one-day, low-titer exception).

Lesions were much more prominent in calves from experiment 1 than calves from any other experiment. Those killed at day 16 (7104, 7108, 7109 and 7600) had more pronounced lesions than the ones killed at day 12 (7102, 7103, 7105 and 7599).

Calves from experiments 2 and 4 which were killed on day 20 or 21 PI had slightly more respiratory tract lesions than did their

partners killed at 12 or 16 days respectively. However, none of the calves of experiment 2, 3 or 4 had as many lesions as did calves of experiment 1 killed at day 12.

Whether the amount of inoculum (10 or 20 ml) or intratracheal versus endobronchial inoculations affected the clinical and pathological characteristics of the disease could not be resolved. It was noted, however, that the surgical procedure for endobronchial inoculation caused a focus of tissue demand for neutrophils and actually interfered with the peripheral blood profile. Since this method did not appear to increase severity of lesions or the shedding pattern of the organism it should be abandoned in experiments of short duration where the effect of mycoplasma on peripheral blood counts is evaluated. Lesions were actually more severe in animals inoculated intratracheally with a minimum of trauma. The endotracheal method should be satisfactory for use in future experiments without the disadvantage of peripheral blood values being altered by wound healing.

Severity of mycoplasma lesions as influenced by dexamethasone treatment could not be fully demonstrated. Variable degrees of lymphoid depletion were, however, evident in the lymphoid tissues of the dexamethasone-treated animals. The three animals with the most extensive lymphoid depletion also developed the most pronounced respiratory lesions associated with high titers of M. dispar (calves 7102, 7109 and 7600). Therefore, it is reasonable to think that dexamethasone enhanced lesion severity.

Peribronchial infiltration with mononuclear cells is considered

a classical and nearly universal feature of mycoplasmal lung disease (Ross, 1978). The lesion appears within a few days after establishment of infection and is assumed to be an immune response to the organism. This assumption is supported by experiments utilizing hamsters in which treatment with antithymocyte serum resulted in reduction of peribronchiolar lymphoid infiltration (Taylor et al. 1974).

The present work demonstrated that dexamethasone had a marked effect in decreasing the lymphocyte population of the thymus (Figure 19A), lymph node (Figure 19B), spleen, tonsil and peribronchial lymphoid tissue (Table 9).

Smith (1977) described similar histologic effects of dexamethasone on lymphoid tissues in cattle. The spleen, lymph nodes and tonsils had variable degrees of lymphocyte depletion. The histopathological observations were obtained in one of a series of experiments in which older cattle were treated for a length of time similar to that of the present work. His calves were killed right after the dexamethasone period (i.e., at the 9th day of a 9 day-period).

The results of the present study indicate that a major lesion which is commonly seen in mycoplasmal pneumonia (peribronchial lymphoid infiltration) was diminished by the blocking effect of dexamethasone on the cell mediated immune response. Thus, a mechanism can be proposed which is similar to anti-lymphocyte studies in hamsters (Taylor et al., 1974). Bronchitis, bronchiolitis and alveolitis, however, were enhanced in the dexamethasone treated calves as will be discussed below.

Investigation of the *in vitro* interaction of mycoplasmas with lymphocytes has been conducted (Cole et al., 1978) and a mitogenic effect of mycoplasmas on lymphocytes was described. Lymphocytes were also alleged to become cytotoxic for certain other host tissues and to produce interferon. Whether or not these mechanisms are operative in the host is not known. The consequences of such actions could be that mitogenic activity of mycoplasmas might induce an infiltration of lymphocytes in the peribronchiolar tissues earlier than might be expected on the basis of immunity alone (Ross, 1978).

Much more work is needed to determine if exacerbation of the disease process would relate to cytotoxic reactions when these cells interact with mycoplasmal antigens as suggested by the *in vitro* studies.

Smith (1977) also contributed some data on the effect of dexamethasone treatment on the blood profile of cattle. His measurements of alternate days within a similar period of treatment are in agreement with the data of the present work. However, continuation of daily measurements in the present experiments allowed observation on the further trends of blood parameters for several days after stopping dexamethasone treatment. There was a normalization of both the total white blood cell counts and neutrophilic compartment by 48 hours after stopping dexamethasone injections. On the other hand, dexamethasone appeared to have a much more prolonged effect on the lymphocytic compartment. The circulating lymphocytes continued in lower numbers as compared with the saline treated calves and there was further lymphoid depletion of thymus, lymph nodes, tonsils, spleen and perhaps

bronchial and bronchiolar associated lymphoid tissue (BALT) in animals necropsied up to 15 days after stopping dexamethasone. This suggests that a complete lymphocytic recovery, if it indeed happens, would take longer than 15 days in 4 to 10-week-old calves treated with dexamethasone for 10 consecutive days.

If the dexamethasone effect on lymphoid tissue and in the animal as a whole could be viewed as a duplication of natural stress, then the immunological repression by dexamethasone would be an adequate method for reproducing the natural disease. If natural stress has similar but milder effects on the lymphoid tissue, some lymphoid depletion should be expected in association with natural cases of respiratory disease where there is a relationship between infectious agents and stress. Therefore, studies on field outbreaks of calf pneumonia should include routine evaluation of lymphoid tissues in order to attempt a correlation of severity of respiratory lesions and the status of the lymphoid system.

Granulomatous lesions were not described in Smith's experiments. This may have been because older calves are not as susceptible to the predisposing dexamethasone influence, or that a longer time is needed for the granulomas to develop. Another more likely explanation for this difference is that the straw used for bedding in this work may have been contaminated by high numbers of fungal spores which, perhaps, were absent from the bedding used by Smith in his experiment. It is worthwhile to note, however, that many workers have demonstrated a high frequency of aspergillus infections among human beings under

prolonged corticosteroid treatments as reviewed by Schwarz (1973).

Respiration rate and rectal temperature were slightly increased by dexamethasone. Changes in other parameters were more pronounced and included significantly increased total white blood cell counts, increased neutrophil counts, decreased lymphocyte counts and decreased plasma protein:fibrinogen ratios.

The increased rectal temperature as an apparent prolonged dexamethasone effect could not be fully explained. A massive amount of neutrophils being destroyed in the tissues after cessation of the dexamethasone effect upon the circulating cells might have been a source of pyrogens. The neutrophil half-life in tissues is not yet well-established. Research should be done in order to clarify this point and help to explain the delayed pyrogenic effect caused by dexamethasone and perhaps by other steroid hormones.

Dexamethasone effects may have slightly impaired evaluation of the morphological changes in the lungs, since a high frequency of granulomatous lesions of fungal origin were observed among the dexamethasone-treated animals in both mycoplasma infected and non-infected groups. The granulomatous lesions did not, however, appear to have interfered much with the surrounding parenchymal tissue. They were excluded from the scoring system adapted to the lung lesions, but their influence on the interstitial lesions may not have been totally avoided.

Lesions of the respiratory tract attributed to infection with M. dispar in this work varied in extent and degree.

Much emphasis has been placed on interstitial thickening and

peribronchial lymphoid hyperplasia as being a direct effect of mycoplasma infections. That can be the case, but a variety of other agents may be responsible for the same type of injuries.

It is the author's opinion that interstitial thickening in routinely prepared lungs has to be evaluated with caution. It is even suspected that the description of this lesion and concomitant "pneumonitis" in some works has been a misinterpretation of artifacts produced by routine tissue processing methods. This supposition is based on evaluation of right and left lungs which were prepared by routine and perfusion methods, respectively. The routinely prepared one-half would indicate a marked, diffuse interstitial thickening in contrast to the normal appearing perfused half. Therefore, perfused lungs (or lobes) supplied sections of higher quality for such an evaluation and perhaps for evaluating other types of lesions.

Actual interstitial thickening as evaluated in perfused lungs was indicated by reduplication of cellular layers and more resistance of the parenchyma to the distensive force of the perfusing fixative.

Diffuse but mild thickening of the alveolar walls was noticed in a majority of the experimental animals. The lesion was more pronounced in calves with high titers of M. dispar and a variety of other lesions but some normal calves had some degree of interstitial thickening.

Lesions such as granulomas, peribronchiolar and perivascular lymphoid accumulations and particular groups of alveoli containing inflammatory cells are easily seen and better evaluated in a perfused

lung. However, the use of perfusion may be disadvantageous in lungs with exudate in the upper airways because the exudate is washed down to smaller airways and alveolar spaces and may cause misinterpretation of the lung lesions.

Relatively pronounced BALT hyperplasia as was seen in infected calves which were treated with saline may not be necessarily linked to mycoplasma infection as has been implied by several workers. Some of the infected calves which were treated with dexamethasone had more severe lung lesions and milder BALT hyperplasia. The follicular arrangement of the BALT was also less pronounced in the dexamethasone treated and infected calves. BALT was prominent to the extent of being classified as BALT hyperplasia in 2 non-infected calves which were treated with saline.

It is the author's opinion that BALT hyperplasia is a non-specific change that can be observed when an animal is exposed to a variety of antigens. It may even reflect a desirable status of immunologic responsiveness. Most animals are exposed to a variety of agents when still young. Some acquire pneumonia and die, without much BALT hyperplasia. Early survival may depend on their intrinsic capacity to deal with these agents. BALT should be considered as an important part of these intrinsic mechanisms. "Cuffing pneumonia", an intensive proliferation of the bronchial associated lymphoid tissue, which several authors tend to attribute to a specific cause, may not be more than an exacerbated immunological reaction as already discussed. If mycoplasmas are the organisms which more persistently colonize the

respiratory mucosa, as they appear to be, they could certainly be assigned as one of the best candidates to incite this immunological reaction. However, it is doubtful that intensive cuffing pneumonia as seen in 3 to 6 month-old-calves in field cases can be reproduced in experiments of short duration like most that have been conducted in cattle unless animals were previously sensitized. On the other hand, it is believed that BALT hyperplasia of a mild nature as reproduced in experiments of short duration may very well be accomplished with other agents.

The lungs of a few animals, mostly of experiment 1, had gross lesions of variable extent and severity. Microscopic changes corresponded to the areas of gross lesions but also extended beyond them. In fact, most of the lungs assigned as normal upon gross inspection had some degree of histological lesions.

All current evidence as based on work with Mycoplasma pneumoniae in hamsters, and in human beings suggests that: (1) Primary infection results in colonization of mucosal epithelial cells of the respiratory tract. (2) A local immune response is generated in response to the attached organisms. (3) Neutrophils and mononuclear inflammatory cells infiltrate as the infection proceeds, peaking towards the end of the second week and then declining as organisms are gradually cleared. Fernald (1978) stated that peribronchial lymphoid infiltrates follow the same time curve during the course of the primary infection. (4) The temporal relationship between peribronchial infiltrates, endobronchial exudates and colonization of the respiratory tract suggest that

all are related to the local immune response. When animals are re-infected, the same sequence of events is repeated in an accelerated fashion characteristic of an anamnestic immune response.

Calves of experiment 1 were all killed at 12 or 16 days PI. These calves had basically the above lesions listed by Fernald (1978) for hamsters and man. Therefore, the idea of reinfection or exacerbation of a pre-existent, inapparent M. dispar infection was reinforced for calves of experiment 1.

Calves of experiments 2, 3 and 4 were considered to have a primary infection. M. dispar colonized the respiratory tract as demonstrated by FAT, culture and electron microscopy. Infiltration of neutrophils and lymphocytes of lamina propria of turbinates, pharynx, trachea, bronchi and bronchioles was consistently demonstrated histologically.

Bronchitis, bronchiolitis and alveolitis of suppurative nature were frequently associated with bronchiectasia and actual pneumonia in addition to the previously discussed interstitial thickening and BALT hyperplasia. All these lesions have been described in connection with mycoplasmal infection from species other than hamsters and man, including cattle (see literature review).

Further association of lesions with evidence of M. dispar in the lung tissues as demonstrated by culture, by FAT and in selected cases by electron microscopy indicated that the lesions were related to M. dispar infection.

The respiratory tract of non-infected calves was essentially normal. There were no gross lesions in the lungs or in any other

segment of the respiratory tract. The development of mild microscopic lung reactions seems to be an unavoidable event in normal conventionally reared animals. Most control animals in this study had some degree of interstitial thickening which was caused by infiltration of round cells and neutrophils. These cells were usually trapped in the alveolar walls. Occasional small groups of alveoli throughout the lung contained increased numbers of alveolar macrophages, neutrophils and fibrin. Also occasionally a bronchiole had a few neutrophils in the lumen which usually was collapsed but without a surrounding parenchymal reaction. Calves 12, 14 and 31 had slightly more pronounced lung changes than the other control calves. The lesions were milder than in any infected calf. Larger airways in the lung, the trachea and the turbinates were essentially normal in most control calves. Neutrophils, if seen in the lamina propria of airways were in very low numbers and were considered normal. They were usually in higher numbers only at the pharyngeal level, which is considered a site of higher antigenic stimulation.

A parallelism between lesions in the tracheas and nasal turbinates could be observed in infected animals. Histopathology, even though from inconsistent pharyngeal sites was performed in tissues from at least 21 calves. The results included a uniformity of the airway lesions. FAT results, microbiological examinations and more limited electron microscopy observations further confirmed involvement of M. dispar in the rather uniform lesions of the airways and of the lung parenchyma.

Microbiology was not applied to the pharynx of all calves. Neither were FAT and electron microscopy applied to all of them. However, the information supplied by all combined work done in pharyngeal tissues allowed the following conclusions: (1) Lesions were more pronounced in the crypt epithelium of the palatine tonsil and the nasopharyngeal mucosa. These were considered to be the harboring sites of mycoplasmas detected by both FAT and culture. Electron microscopy revealed mycoplasma only in the ciliated epithelium of the nasopharynx, however. (2) The frequently high titers of pharyngeal isolations of M. dispar, electron microscopic demonstrations of mycoplasma, and positive fluorescent antibody tests in several calves, may very well be an important indicator of the mechanism of shedding of the organism and its spread from animal to animal in nature. Further work should be done to determine the validity of such a line of thought. (3) As future work involving the respiratory tract is planned, special care should be exercised in regard to specific sites for tissue harvesting at the pharyngeal level. The palatine tonsil usually supplies suitable material for evaluation of both the oropharyngeal mucosa and its lymphoid tissue. As far as the nasopharynx is concerned, more than one specific site should be selected so as to have at least one section with submucosal lymphoid tissue (nasopharyngeal tonsil) and one without it.

To serve multiple purposes such as microbiological isolations, fluorescent antibody techniques, electron microscopy and histopathology the site should be consistent and properly identified to allow meaningful conclusions.

Nasal swabs taken from all 24 calves were negative on culture at the time they entered an experiment. However, all 8 animals in experiment 1 including intentionally exposed and non-exposed calves started shedding M. dispar which was detected in nasal swabs at about one week after experimental exposure. The shedding continued throughout the rest of the experimental period. The organism was recovered from the respiratory tract, demonstrated in tissues by fluorescent antibody technique and in a few selected tissues by electron microscopy. They also had lesions compatible with an active infection.

The source of contamination of the 4 calves in the DEXONLY and CONTROL groups of experiment 1 could not be satisfactorily explained and there are multiple possibilities to be analyzed.

Thomas and Smith (1972) reported the recovery of several mycoplasma species, including M. dispar from the respiratory tract of calves as young as 1 to 2 days-old.

Experiment 1 utilized 8 calves which came from a private farm when they were two to two and one-half weeks old. In the remaining three experiments, sixteen 1-day-old calves from the Iowa State University Research Farm were used.

The fact that those 8 calves had been on the farm for a long enough time to be contaminated makes it reasonable to see that as a possibility. Coughing, even though very transient, was observed in most of the experimental animals at the time of inoculating the medium into the trachea and for variable amounts of time thereafter. Such a natural reflex and perhaps slight changes in the respiratory ciliary

activity may have been the necessary stimulus for a low, undetectable burden of mycoplasma to multiply and become established or spread. Animals intentionally exposed to an additional burden of mycoplasma in the inoculum may have had this as an extra contribution to the further clinical, microbiological and pathological observations such as in calf 7600.

In contrast, CONTROL and DEXONLY calves from the other experiments remained uninfected for the length of the experimental period and, at necropsy, none of their tissues yielded M. dispar or gave positive results with FAT or electron microscopy. A significant tissue reaction, as evaluated histologically could not be demonstrated. Therefore, the process of obtaining newborn calves and raising them in isolation units appears to be a suitable way of selecting calves for a respiratory disease project involving mycoplasma. Older animals might have had enough time to be contaminated by mycoplasmas in their original environment.

If the possibility of transmission of infection by animal caretakers is considered, then one has to consider that transmission in nature may be more easily accomplished than has been suspected. Whether this is the best explanation or not, studies should be conducted to determine the spread of M. dispar from infected to non-infected premises in order to seek epidemiological information on the natural behavior of this organism.

The microbiological and pathological observations obtained in the present work demonstrated a definite relationship between M. dispar

infection and the presence of mild disease of the upper and lower respiratory tract.

The mechanisms by which the organism caused this range of mild lesions could not be determined. As a matter of fact, it has not yet been determined for any of the other better known mycoplasmoses. Researchers appear to have emphasized the lymphocytic response of the inflammation but avoid discussing the other aspects. It is the author's opinion that a mild neutrophilic response at least at the tissue level in the early phase of the disease is an essential part of the infection. Because of this pattern the disease is impossible to differentiate on the basis of histological observations alone. Neutrophilic chemotaxis and, to a lesser extent, lymphocytic chemotaxis are subjects exhaustively studied but not yet fully understood. There is a possibility that the mycoplasma interaction with the cilia of the respiratory epithelium (perhaps the better understood aspect of mycoplasma infection up to this point) is enough stimulus to release some sort of chemotactic substance. This would cause infiltration of low numbers of neutrophils initially which would later become responsible for further attraction of more neutrophils and, in a later phase, lymphocytes.

Since animals on dexamethasone treatment had less BALT and interstitial thickening than did saline-treated animals, the interstitial thickening appears to be governed by the same stimulus which causes BALT hyperplasia.

The disease produced was not severe enough to disturb the clinical status of most of the infected animals but certainly has to be

considered as a subclinical disturbance of respiratory structures, mainly cilia. Ciliary changes, especially loss of cilia, are known to reduce function of the mucociliary blanket mechanism. So, it is reasonable to look at this level of morphological change as a possible predisposing influence to further noxious agents such as viruses and bacteria. These changes may also be early lesions of a disease that would require a longer time to develop to the extent they are seen in field situations and described in older animals.

Future research should be directed toward viral and bacterial inoculations in calves pre-exposed to M. dispar for a few to several days. Experiments of longer duration should also be undertaken to determine the prolonged effect of M. dispar in more chronic disease.

SUMMARY

Infection with Mycoplasma dispar was established in seventeen 4 to 10-week-old Holstein-Friesian calves (infected). Seven calves of the same breed and age range served as controls (non-infected).

Daily dexamethasone or saline injections were given to calves during a 10-day-period. The calves were inoculated with M. dispar (infected) or sterile medium (non-infected) midway through the dexamethasone or saline series. Nine of the 17 infected calves were saline treated and 8 were dexamethasone treated while 3 of the 7 non-infected calves were saline treated and 4 were dexamethasone treated. Calves were necropsied at 11, 12, 16, 20 and 21 days post infection.

Clinical signs of respiratory infection were mild to absent except in one M. dispar infected animal which had clinical pneumonia. Total white blood cell counts, absolute numbers of neutrophils, respiration rate, and temperature were significantly increased by administration of dexamethasone. Lymphocyte counts and plasma protein: fibrinogen ratio were significantly decreased. Respiration rate was decreased by infection with M. dispar, and rectal temperature was slightly increased. No significant changes were observed in blood parameters as a result of M. dispar infection.

The shedding pattern of M. dispar as determined by culture of nasal swabs varied considerably from animal to animal. Some animals which did not shed the organism, however, were actually infected as determined by culture and fluorescent antibody tests at necropsy.

The group of 17 infected calves included 11 animals which shed M. dispar as well as 6 in which the organism was identified only at necropsy. The presence of mycoplasma in the respiratory tract was demonstrated in selected cases by electron microscopy.

All M. dispar infected calves had some degree of respiratory lesions which involved the upper respiratory tract, the airways in general, and the lung parenchyma. Dexamethasone treatment caused slight enhancement of the lesions. Control calves did not have significant lesions. Turbinate, pharyngeal, and tracheal lesions in infected calves consisted of variable degrees of mononuclear inflammatory cell and neutrophil infiltration of the lamina propria.

Lung lesions included variable degrees of neutrophil infiltration of the walls and lumina of bronchi, bronchioles and alveoli. The parenchymal tissue was thickened by infiltrations of mononuclear inflammatory cells in addition to neutrophils. Peribronchial, peribronchiolar and perivascular accumulations of lymphoid tissue were also consistently present. Slight to considerable clumping and loss of cilia were found in the epithelium from the turbinates down to the small bronchioles.

The lesions were observed in calves necropsied as early as 11 days post infection but were more pronounced in animals necropsied 16 or more days post infection.

Numerous minute, multifocal granulomatous lesions of fungal origin were observed in most of the dexamethasone-treated calves but not in calves which did not receive dexamethasone.

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APPENDIX

Table A1. Daily means of respiration rates per minute for dexamethasone and saline treated calves

Days	Dexamethasone[a]		Saline	
	Morning Respiration	Evening Respiration	Morning Respiration	Evening Respiration
-7	35.3	47.7	38.0	45.7
-6	31.7	39.7	35.7	40.3
-5	31.0	34.0	35.7	37.3
-4	31.1	31.3	32.0	30.4
-3	31.9	33.7	29.3	29.0
-2	31.3	36.4	30.8	30.6
-1	36.3	40.4	38.0	36.9
0[b]	40.3	39.6	33.8	39.5
1	36.8	43.9	36.6	34.8
2	40.4	43.0	39.7	39.7
3	43.7	40.7	34.0	37.0
4	46.4	41.8	38.2	41.4
5	46.8	42.7	39.5	35.7
6	41.1	46.6	36.2	38.1
7	41.5	38.3	33.7	38.4
8	41.7	42.6	40.7	42.1
9	43.3	38.9	38.7	38.4
10	43.0	41.7	38.4	40.5
11	45.1	48.9	36.8	45.8
12	44.8	56.1	40.2	46.9
13	49.5	53.1	49.4	44.9
14	55.3	55.4	45.3	45.0
15	48.9	54.5	38.4	44.3
16	50.4	55.8	42.3	44.2
17	53.6	55.4	44.6	46.4
18	50.2	55.2	47.8	45.0
19	54.0	51.6	42.8	45.4
20	49.8	43.0	40.2	35.5
21	33.5	ND[c]	49.5	ND

[a]Dexamethasone was administered on day -7 to day 5.

[b]Inoculation of calves with Mycoplasma dispar or sterile medium.

[c]ND = Not done, calves killed after morning observation.

Table A2. Daily means of rectal temperatures (^{o}C) for dexamethasone and saline treated calves

Days	Dexamethasone[a]		Saline	
	Morning Temperature	Evening Temperature	Morning Temperature	Evening Temperature
-7	39.30	39.03	39.27	39.07
-6	38.93	38.97	38.77	38.53
-5	38.97	39.17	38.73	38.97
-4	39.07	39.23	38.95	38.91
-3	39.06	39.17	38.88	39.05
-2	39.23	39.25	39.03	39.23
-1	39.41	39.32	39.17	39.27
0[b]	39.35	39.25	39.15	39.43
1	39.38	39.38	39.17	39.25
2	39.19	39.45	39.18	39.33
3	39.37	39.37	39.07	39.29
4	39.23	39.35	39.15	39.37
5	39.22	39.36	39.13	39.29
6	39.24	39.67	39.18	39.27
7	39.52	39.57	39.00	39.18
8	39.40	39.35	39.18	39.18
9	39.20	39.49	39.03	39.20
10	39.37	39.53	39.04	39.20
11	39.31	39.61	39.03	39.24
12	39.66	39.90	39.16	39.21
13	39.74	39.66	39.30	39.36
14	39.54	39.47	39.21	39.15
15	39.31	39.49	38.97	39.27
16	39.43	39.26	39.13	39.08
17	39.16	39.30	38.98	39.28
18	39.30	39.18	39.22	39.32
19	39.30	39.20	39.00	39.10
20	39.20	39.00	39.06	38.90
21	38.85	ND[c]	38.90	ND

[a]Dexamethasone was administered on day -7 to day 5.

[b]Inoculation of ccalves with Mycoplasma dispar or sterile medium.

[c]ND = Not done, calves killed after morning observation.

Table A3. Daily means of respiration rates per minute of Mycoplasma dispar infected and non-infected calves

Days	Infected		Non-infected	
	Morning Respiration	Evening Respiration	Morning Respiration	Evening Respiration
-7	33.50	41.75	43.00	56.50
-6	32.50	38.00	36.00	44.00
-5	35.00	34.75	30.00	37.50
-4	27.71	28.59	40.86	36.43
-3	27.00	28.88	39.43	37.43
-2	28.53	31.94	37.14	37.29
-1	36.71	35.94	38.29	45.29
0[a]	37.12	38.47	36.86	42.14
1	33.88	35.71	43.57	48.29
2	37.06	37.47	47.29	50.86
3	35.00	37.94	48.14	41.14
4	39.29	39.47	49.57	46.86
5	41.35	38.47	47.57	41.00
6	37.53	39.59	41.29	49.00
7	36.06	37.24	41.29	41.00
8	38.59	38.12	47.57	52.57
9	39.41	37.06	44.71	42.57
10	38.76	36.47	45.43	52.29
11	38.56	47.13	46.00	47.50
12	40.87	49.73	46.00	55.40
13	50.09	48.91	48.00	49.20
14	47.91	50.09	55.40	50.40
15	41.73	45.73	47.80	57.40
16	43.45	51.00	52.60	49.00
17	48.20	49.00	50.00	52.80
18	49.80	47.20	48.20	53.00
19	46.80	46.20	50.00	50.80
20	42.20	41.00	47.80	37.50
21	43.00	ND[b]	40.00	ND

[a] Inoculation with Mycoplasma dispar (infected) or sterile medium (non-infected).

[b] ND = Not done, calves killed after morning observation.

Table A4. Daily means of rectal temperatures (^{o}C) for Mycoplasma dispar infected and non-infected calves

Days	Infected		Non-infected	
	Morning Temperature	Evening Temperature	Morning Temperature	Evening Temperature
-7	38.35	39.13	39.15	38.90
-6	38.85	38.80	38.85	38.65
-5	38.97	39.13	38.60	38.95
-4	38.85	39.02	39.39	39.19
-3	38.95	39.06	39.03	39.24
-2	39.14	39.28	39.11	39.14
-1	39.26	39.31	39.36	39.26
0[a]	39.18	39.34	39.43	39.34
1	39.24	39.36	39.37	39.21
2	39.19	39.44	39.17	39.27
3	39.18	39.31	39.33	39.39
4	39.15	39.37	39.29	39.34
5	39.18	39.38	39.16	39.20
6	39.26	39.52	39.09	39.34
7	39.26	39.48	39.26	39.14
8	39.23	39.32	39.44	39.14
9	39.11	39.46	39.13	39.07
10	39.31	39.52	38.96	38.99
11	39.14	39.44	39.25	39.32
12	39.39	39.61	39.38	39.44
13	39.58	39.56	39.38	39.40
14	39.38	39.28	39.36	39.38
15	39.17	39.41	39.08	39.32
16	39.26	39.16	39.30	39.18
17	39.10	39.28	39.04	39.30
18	39.40	39.28	39.12	39.22
19	39.10	39.10	39.20	39.20
20	39.24	39.00	39.02	38.90
21	38.90	ND[b]	38.85	ND

[a] Inoculation with Mycoplasma dispar (infected) or sterile medium (non-infected).

[b] ND = Not done, calves killed after morning observation.

Table A5. Daily means of total white blood cell counts for dexamethasone and saline treated calves

Days	Dexamethasone[a]	Saline
-7	13200	12067
-6	31033	11033
-5	31767	11067
-4	12542	8892
-3	25717	9033
-2	25725	9058
-1	21336	8658
0[b]	22683	8954
1	17792	9558
2	17883	8908
3	17908	9058
4	18917	8902
5	20255	8500
6	18225	8313
7	12717	8275
8	11858	9058
9	10936	9533
10	10825	8775
11	11172	8810
12	10830	9050
13	11700	8657
14	10387	10174
15	9200	8213
16	8620	9077
17	11860	8820
18	8141	8960
19	8480	8680
20	8520	8340
21	11800	12500

[a]Dexamethasone was administered on day -7 to day 5.

[b]Inoculation of calves with Mycoplasma dispar or sterile medium.

Table A6. Daily means of absolute numbers of segmented neutrophils and lymphocytes for dexamethasone and saline treated calves

	Dexamethasone[a]		Saline	
Days	Neutrophils	Lymphocytes	Neutrophils	Lymphocytes
-7	7386	5441	4055	7034
-6	26782	3209	3331	6967
-5	27332	3242	3705	7238
-4	7907	4147	3967	4510
-3	21479	3018	3739	4955
-2	20767	3458	3651	5053
-1	16050	3554	3320	4982
0[b]	19263	2691	3991	4705
1	13912	3199	4549	5493
2	13870	3228	3151	5457
3	14224	2964	3341	5422
4	15332	3001	3366	5263
5	16994	2444	3188	5029
6	14532	3035	3346	4726
7	9871	2390	3739	4357
8	8509	3030	4139	4700
9	7358	3143	4426	4812
10	7328	3140	4045	4469
11	7209	3766	3400	5169
12	6960	3522	3282	5393
13	6687	4887	3256	5254
14	5849	4334	4078	5821
15	5475	3498	3129	4760
16	5033	3391	3675	5079
17	6464	4945	2689	5727
18	4731	3204	2969	5648
19	5404	2661	2846	5229
20	4966	3123	2783	5069
21	8143	3311	3220	8460

[a]Dexamethasone was administered on day -7 to day 5.

[b]Inoculation of calves with *Mycoplasma dispar* or sterile medium.

Table A7. Daily means of plasma protein:fibrinogen ratios for dexamethasone and saline treated calves

Days	Dexamethasone[a]	Saline
-7	13.48	9.50
-6	10.92	12.44
-5	10.26	16.22
-4	9.73	10.79
-3	11.42	11.31
-2	11.41	13.76
-1	8.00	11.80
0[b]	7.75	15.00
1	7.83	11.06
2	8.02	11.05
3	6.58	9.91
4	6.43	10.87
5	6.39	10.42
6	12.95	24.25
7	6.84	12.73
8	6.71	13.88
9	7.02	11.35
10	6.52	15.38
11	6.98	11.80
12	8.22	13.33
13	7.57	13.44
14	8.71	14.69
15	8.92	17.99
16	9.26	11.57
17	12.36	18.14
18	20.01	16.44
19	12.85	13.70
20	14.50	14.97
21	13.04	16.25

[a]Dexamethasone was administered on day -7 to day 5.

[b]Inoculation of calves with Mycoplasma dispar or sterile medium.

Table A8. Daily means of total white blood cell counts for Mycoplasma dispar infected and non-infected calves

Days	Infected	Non-infected
-7	11975	13950
-6	20875	21350
-5	21225	21800
-4	10500	11243
-3	17853	16214
-2	17171	17929
-1	13318	17929
0[a]	13497	21457
1	13029	15243
2	12912	14571
3	12723	15329
4	13201	15629
5	13587	15343
6	13256	13300
7	11005	9257
8	10823	9571
9	10118	10400
10	9770	9871
11	10423	8567
12	9963	9583
13	10580	9680
14	10426	9960
15	8645	8840
16	8705	9164
17	8240	12440
18	8942	8159
19	9280	7880
20	9020	7840
21	13300	11000

[a]Inoculation with Mycoplasma dispar (infected) or sterile medium (non-infected).

Table A9. Daily means of absolute numbers of segmented neutrophils and lymphocytes for Mycoplasma dispar infected and and non-infected calves

	Infected		Non-infected	
Days	Neutrophils	Lymphocytes	Neutrophils	Lymphocytes
-7	5361	5880	6441	6951
-6	14936	5288	15298	4687
-5	14584	5981	17387	3759
-4	5343	4695	7380	3438
-3	12887	4106	11933	3695
-2	11453	4571	14046	3490
-1	7680	4560	13358	3703
0[a]	9140	3793	17666	3469
1	8692	4309	10538	4437
2	7984	4328	9790	4377
3	7932	4272	10848	4000
4	8793	3879	10700	4747
5	8955	4013	11703	3291
6	8840	3926	9178	3770
7	7255	3448	5715	3191
8	6408	4082	6119	3340
9	5582	4153	6391	3694
10	5777	3641	5468	4202
11	5671	4508	3947	4468
12	5147	4453	4446	4781
13	5108	5361	5041	4453
14	5009	5203	4863	4801
15	4137	4212	4664	3946
16	4288	4164	4500	4391
17	3679	4246	5473	6426
18	3901	4804	3799	4047
19	4568	4091	3681	3798
20	4524	3909	3225	4283
21	6468	6216	4895	5555

[a]Inoculation with Mycoplasma dispar (infected) or sterile medium (non-infected).

Table A10. Daily means of plasma protein:fibrinogen ratios for Mycoplasma dispar infected and non-infected calves

Days	Infected	Non-infected
-7	10.86	12.75
-6	10.40	14.25
-5	11.94	15.83
-4	10.32	10.12
-3	11.66	10.65
-2	12.95	11.71
-1	9.92	10.12
0[a]	11.85	10.24
1	8.59	11.50
2	9.37	9.92
3	7.81	9.30
4	8.44	9.15
5	8.17	9.23
6	22.52	9.09
7	10.10	9.04
8	9.83	11.42
9	9.60	8.56
10	11.63	9.31
11	9.18	10.40
12	10.50	12.38
13	9.81	11.31
14	12.40	10.15
15	10.20	20.62
16	9.74	11.90
17	13.36	17.14
18	14.20	22.25
19	14.04	12.51
20	16.26	13.21
21	15.87	13.42

[a]Inoculation with Mycoplasma dispar (infected) or sterile medium (non-infected).